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<b>(54) Title:</b> GENETIC SEQUENCES CONFERRING DISEASE RESISTANCE IN PLANTS AND USES THEREFOR  <b>(57) Abstract</b> <p>The present invention relates generally to genetic sequences, and more particularly to genetic sequences which confer or otherwise facilitate disease resistance in plants such as against rust and mildew. The present invention further provides for transgenic plants carrying the subject genetic sequences enabling the generation of disease resistant plants. The present invention is particularly useful for developing disease resistance in crop varieties.</p>		

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**GENETIC SEQUENCES CONFERRING DISEASE  
RESISTANCE IN PLANTS AND USES THEREFOR**

5 The present invention relates generally to genetic sequences, and more particularly to genetic sequences which confer or otherwise facilitate disease resistance in plants such as against rust and mildew. The present invention further provides for transgenic plants carrying the subject genetic sequences enabling the generation of disease resistant plants. The present invention is particularly useful for developing  
10 disease resistance in crop varieties.

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are  
15 defined following the bibliography.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers  
20 but not the exclusion of any other element or integer or group of elements or integers.

The rapidly increasing sophistication of recombinant DNA technology is greatly facilitating the efficacy of commercially important agricultural processes. Of  
25 particular concern is the effect of plant diseases on the efficacy of these agricultural processes. Plant diseases and particularly diseases in crop plants represent a major contributing factor in crop losses capable of causing economically significant down turn in productivity per square metre. The development, therefore, of disease resistant plants is an important goal in agricultural and horticultural research.

Major genes conditioning resistance to plant diseases have been investigated extensively in agriculture. Of particular economic importance are genes controlling resistance to rust and mildew. Rust is an especially significant problem amongst  
5 broad acre crops such as wheat, barley and cereal grains and is caused by infection with a class of fungi known as the *Basidiomycetes*. Although rust resistance genes are a potentially invaluable genetic resource in agriculture, the molecular basis of major gene resistance is unknown and, until the present invention, genes conferring rust or mildew resistance had not been cloned.

10

Genetic analysis indicates that rust resistance genes control specific recognition of the products of rust avirulence genes. In developing disease resistance varieties using disease resistance genes, plant breeders deploy resistance genes that match the avirulence genes present in the local strains of the pathogens. The pathogen  
15 populations are dynamic and frequently new pathogenic strains arise by any number of means such as by mutation, recombination or accidental or natural introduction of new pathogenic strains. The existing disease resistant varieties then become susceptible to the new pathogenic strains. Plant breeders are then forced to develop new disease resistant varieties. At present, breeders use resistance genes that exist in  
20 either wheat or its relatives as their pool of new genes.

The cloning of a resistance gene is the first step in understanding the basis of resistance gene action and in particular the specificity control mechanism. Ellis *et al* (1) have described the development of a transposon tagging system to isolate rust  
25 resistance genes from flax (linseed, *Linum usitatissimum*). However, despite the general effectiveness of the methodology, the isolation of mutant flax plants possessing a tagged resistance gene has not been a straight forward procedure (2).

In accordance with the present invention, genetic sequences conferring rust resistance  
30 have been cloned from flax. The cloning of these sequences provides the means of generating transgenic plants with *de novo*, increased or otherwise enhanced rust resistance. The present invention also permits the screening through genetic or

immunological means similar rust resistance genes in other plants for use in developing or enhancing rust resistance in commercially and economically important species. Furthermore, the application of knowledge of the molecular basis behind resistance gene action and the specificity thereof offers the potential of a new source  
5 of genes produced by a variety of recombinant techniques. These new genes with altered disease resistance specificities are referred to herein as "modular resistance genes".

Accordingly, one aspect of the present invention comprises an isolated nucleic acid  
10 molecule comprising a sequence of nucleotides which confers or otherwise facilitates disease resistance in a plant. More particularly, the disease resistance is rust resistance. The present invention extends, however, to resistance to obligate biotrophic pathogens including but not limited to fungi, viruses and nematodes.

15 Another aspect of the present invention is directed to a nucleic acid molecule which comprises a sequence of nucleotides corresponding or complementary to the nucleotide sequence set forth in Figure 1 (SEQ ID NO:1) or having at least 45% similarity to all or part thereof and wherein said nucleic acid molecule confers or otherwise facilitates rust resistance in a plant.

20

In a related aspect of the present invention there is provided a nucleic acid molecule which comprises a sequence of nucleotides encoding or complementary to a sequence of nucleotides encoding the amino acid sequence set forth in Figure 1 (SEQ ID  
25 NOs:2 to 5) or having at least 45% similarity to all or part thereof and wherein said nucleic acid molecule confers or otherwise facilitates rust resistance in a plant

Preferably, the percentage similarity to the sequence set forth in SEQ ID NO:1 or  
SEQ ID NOs:2 to 5 is at least 50%. Even more preferably, the percentage similarity is at least 60%. Still more preferably, the percentage similarity is at least 65%. Yet  
30 still more preferably, the percentage similarity is at least 80-90% including at least 91% or 93% or 95%.

For the purposes of nomenclature, the sequences in Figure 1 relate to the "L6" allele of the disease resistance gene L which controls host resistance to flax rust races that carry the corresponding avirulence gene AL6. However, the present invention extends to other alleles of the gene or alleles of the M gene. Accordingly, a related  
5 embodiment of the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a peptide, polypeptide or protein wherein said peptide, polypeptide or protein is capable of interacting with an avirulence gene product on a flax rust. Generally, when the rust resistance gene is the L6 allele, the corresponding  
10 avirulence gene is AL6. A similar nomenclature applied to the other alleles of the L gene, for example, the L2 and L10 alleles. Their corresponding avirulence genes are AL2 and AL10, respectively.

A further aspect of the present invention contemplates a nucleic acid molecule which  
15 confers or otherwise facilitates rust resistance in a plant and which is capable of hybridising under at least low stringency conditions to the nucleic acid molecule defined in SEQ ID NO:1.

For the purposes of defining the level of stringency, reference can conveniently be  
20 made to Sambrook *et al* (3), which is herein incorporated by reference, where the washing steps at pages 9.52-9.57 are considered high stringency. A "low" stringency is defined herein as being in 0.1-0.5% w/v SDS at 37-45°C for 2-3 hours. Depending on the source and concentration of nucleic acid involved in the hybridisation, alternative conditions of stringency may be employed such as  
25 "medium" stringent conditions which are considered herein to be 0.25-0.5% w/v SDS at  $\geq 45^{\circ}\text{C}$  for 2-3 hours or "high" stringent conditions as disclosed by Sambrook *et al* (3).

Although not intending to limit the present invention to any one theory or mode of  
30 action, it is proposed that the genetic sequences of the present invention are necessary for specific recognition of the products of rust avirulence genes. Accordingly, the genetic sequences are useful in enhancing existing rust resistance genes, providing *de*

*novo* the required specific recognition of rust avirulence gene products or being introduced together with rust avirulence genes on, for example, a single genetic cassette. Accordingly, these aspects of the invention are covered by the expression "conferring or otherwise enhancing rust resistance" or other similar expression.

5

The present invention is particularly directed to resistance conferring or facilitating genetic sequences from flax plants and from its relative *Linum marginale*. However, the subject invention clearly contemplates other sources of rust resistance genes such as but not limited to other species of *Linum*, soybeans, sunflowers and cereals  
10 amongst other plants including wild varieties of wheat, barley and maize.

The genetic sequences conferring or otherwise facilitating rust resistance may correspond to the naturally occurring sequence or may differ by one or more nucleotide substitutions, deletions and/or additions. Accordingly, the present  
15 invention extends to rust resistance genes and any functional alleles mutants, derivatives, parts, fragments, homologues or analogues thereof or non-functional molecules but which are at least useful as, for example, genetic probes or in the generation of immunologically interactive recombinant molecules. The present invention further extends to the promoter region from the rust resistance genes such  
20 as from L6, L2 or L10. A preferred promoter is from L6. Such promoters may be useful in driving expression of transgenes.

Examples of alleles of the flax rust resistance gene L include, but are not limited to L6, L2 and L10 although all alleles of the L locus are encompassed by the present  
25 invention. The present invention also extends to alleles of the M locus.

The present invention further contemplates recombinant or synthetic rust resistance gene products, such as the products of the L6, L2 or L10 alleles of the L resistance gene. A particularly preferred recombinant or synthetic rust resistance gene product  
30 is from the L6 gene. The L6 protein has the following features; an amino terminal half containing a nucleotide binding site (NBS) consisting of several motifs including the P-loop and further downstream, a kinase-2 motif (14). In L6, the kinase-2

sequence is ILVVLDDVD (SEQ ID NO:13) and more generally, XXXXDDX where X=L,I,V,F (using single amino acid nomenclature defined below). The C-terminal half of the polypeptide is a leucine rich region consisting of approximately 17% leucine residues. Within this region, stretches of residues appear where leucine is reiterated LXXL or LXL where X = any residue. These sequences are similar to the leucine-rich repeat sequences of 25-30 residues that are found in many proteins that form protein-protein interactions. More particularly in the L6 product, the leucine rich region of L6 consists of two parts with the C-terminal part of the region consisting of two direct leucine rich repeats of 146 and 149 amino acids that are 74% identical.

The above mentioned functional mutants, derivatives, parts, fragments, homologues and analogues of are referred to herein as "rust resistance-like genes" or "rust resistance genetic sequences". Reference herein to "genes" is to be taken in its broadest context and includes a classical genomic gene as well as mRNA or cDNA corresponding to the coding regions (i.e. exons) of the gene. The term "gene" is also used to describe synthetic or fusion molecules encoding all or part of a functional product. Preferred rust resistance-like genes are derived from a naturally occurring rust resistance gene by standard recombinant techniques. Generally, a rust resistance gene may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or additions. Nucleotide insertional derivatives of the rust resistance gene of the present invention include 5' and 3' terminal fusions as well as intra-sequence insertions of single or multiple nucleotides. Insertional nucleotide sequence variants are those in which one or more nucleotides are introduced into a predetermined site in the nucleotide sequence although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterised by the removal of one or more nucleotides from the sequence. Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide inserted in its place. Such a substitution may be "silent" in that the substitution does not change the amino acid defined by the codon. Alternatively, substituents are designed to alter one amino acid for another similar acting amino acid. Typical substitutions are those made in



accordance with the following:

Suitable residues for amino acid substitutions		
	<u>Original Residue</u>	<u>Exemplary Substitutions</u>
5	Ala	Ser
	Arg	Lys
	Asn	Gln; His
	Asp	Glu
	Cys	Ser
10	Gln	Asn
	Glu	Asp
	Gly	Ala
	His	Asn; Gln
	Ile	Leu; Val
15	Leu	Ile; Val
	Lys	Arg; Gln; Glu
	Met	Leu; Ile
	Phe	Met; Leu; Tyr
	Ser	Thr
20	Thr	Ser
	Trp	Tyr
	Tyr	Trp; Phe
	Val	Ile; Leu

- 25 In a particularly preferred embodiment, the rust resistance genetic sequences or like genetic sequences are employed to identify and isolate similar genes from other plants. According to this embodiment, there is contemplated a method for identifying a rust resistance genetic sequence or rust resistance-like genetic sequence in a plant, said method comprising contacting genomic DNA or cDNA isolated from
- 30 said plant with a hybridisation effective amount of a genetic sequence conferring or otherwise rust resistance or part thereof and then detecting said hybridisation.

Preferably, the latter mentioned genetic sequence is from flax or similar plant such as a *Linum* species. In a most preferred embodiment, the latter genetic sequence is as set forth in SEQ ID NO:1 or corresponds to a probe such as defined in Figure 2 (SEQ ID NO:11).

5

Preferably, the latter genetic sequence is labelled with a reporter molecule capable of giving an identifiable signal (e.g. a radioisotope such as  $^{32}\text{P}$  or  $^{35}\text{S}$  or a biotinylated molecule).

- 10 Preferably, the plant to be screened is a flax, a *Linum* species or a grain crop plant such as wheat, barley, maize, rye, lupins or rice and/or wild varieties of same.

Alternatively, the plant is screened using antibodies to a recombinant product of a rust resistance gene. According to a first aspect of this embodiment, the present  
15 invention provides for the expression of the subject genetic sequence in a suitable host (e.g. a prokaryote or eukaryote) to produce full length or non-full length recombinant rust resistance gene products. The only requirement being that the recombinant products are immunologically interactive with antibodies to all or part of a rust resistance gene product. The immunological screening for rust resistance gene  
20 products may, in some circumstances, be more suitable than a genetic screening procedure.

Another aspect of the present invention is, therefore, directed to antibodies to a recombinant rust resistance gene product or part or fragment thereof. Such  
25 antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to a rust resistance gene product or may be specifically raised to a recombinant rust resistance gene product. In the case of the latter, the rust resistance gene product may first need to be associated with a carrier molecule. Alternatively, fragments of antibodies may be used such as Fab fragments.  
30 Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A "synthetic antibody" is considered herein to include fragments and hybrids of antibodies. In additions, antibodies may be raised to

fragments or derivatives of the rust resistance gene product such as oligopeptides derived from or based on the product of, for example, the L6 gene. The antibodies and/or the recombinant rust resistance gene products of the present invention are particularly useful for the immunological screening of rust resistance gene products in various plants, in monitoring expression of rust resistance genetic sequences in transgenic plants and as a proprietary tagging system.

In one embodiment, specific antibodies are used to screen for rust resistance gene products in plants. Techniques for the assays contemplated herein are known in the art and include, for example, sandwich assays and ELISA.

It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal or fragments of antibodies) directed to the first mentioned antibodies discussed above. Both the first and second antibodies may be used in detection assays or a first antibody may be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of a recombinant rust resistance gene product.

Both polyclonal and monoclonal antibodies are obtainable by immunisation with a recombinant rust resistance gene product and either type is utilisable for immunoassays. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal with an effective amount of recombinant rust resistance gene product, or antigenic or immunointeractive parts thereof, collecting serum from the animal and isolating specific sera by any of the known immunoadsorbent techniques. Although antibodies produced by this method are utilisable in virtually any type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production

derived by fusing an immortal cell line and lymphocytes sensitised against the immunogenic preparation can be done by techniques which are well known to those who are skilled in the art (see, for example, references 4, 5 and 6).

- 5 The presence of a rust resistance gene product in a plant or more commonly plant extract may be accomplished in a number of ways such as by Western blotting and ELISA procedures. A wide range of immunoassay techniques are available as can be seen by reference to US Patent Nos. 4,016,043, 4, 424,279 and 4,018,653. These, of course, includes both single-site and two-site or "sandwich" assays of the non-  
10 competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target.

Sandwich assays are among the most useful and commonly used assays and are favoured for use in the present invention. A number of variations of the sandwich  
15 assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antibody is immobilised on a solid substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen complex, a second antibody specific to the  
20 antigen, labelled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labelled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal produced by the reporter molecule.

25

In this case, the first antibody is raised to a recombinant rust resistance gene product and the antigen is a rust resistance gene product in a plant.

The results may either be qualitative, by simple observation of the visible signal, or  
30 may be quantitated by comparing with a control sample containing known amounts of hapten. Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody.

These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent. In accordance with the present invention the sample is one which might contain rust resistance gene product and include crude or purified plant extract such as extracts of leaves, roots and stems.

5

In the typical forward sandwich assay, a first antibody raised against a recombinant rust resistance gene product is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or  
10 polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking, covalent binding or physically adsorption, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then  
15 added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes) and under suitable conditions (e.g. 25°C) to allow binding of any antigen present in the sample to the antibody. Following the incubation period, the reaction locus is washed and dried and incubated with a second antibody specific for a portion of the first antibody. The second antibody is linked to a reporter molecule  
20 which is used to indicate the binding of the second antibody to the hapten.

An alternative method involves immobilising the target molecules in the biological sample and then exposing the immobilised target to specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target  
25 and the strength of the reporter molecule signal, a bound target may be detected by direct labelling with the antibody. Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

30

By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognised, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody-hapten complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of hapten which was present in the sample. The term "reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state to excitability in the

molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in enzyme immunoassays (EIA), the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex.

After washing off the unbound reagent, the remaining tertiary complex is then

- 5 exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

10

It will be readily apparent to the skilled technician how to vary the above assays and all such variations are encompassed by the present invention.

The present invention is particularly described with reference to the flax rust

- 15 resistance gene L and its alleles L6, L2 and L10. This is done, however, with the understanding that the subject invention extends to a range of resistance genes and alleles thereof for rust and other pathogens. In fact, the present invention extends to a resistance gene characterised by said gene encoding a product having at least one leucine rich region. Preferably, the leucine rich region is a leucine rich repeat at the
- 20 3' end of the molecule and has at least 60% similarity to each other more preferably at least 70% similarity and even more preferably at least 80% similarity. Still more preferably, the leucine rich region corresponds to the following amino acid sequence:

PDLIELLPCELGGQTVV.VPSMAELTIRDCPRLEVGP MIRSLPKF PMLKK  
25 LDLAVANITKEEDLDAIGSLEELVSLELELDDTSSGIERIVSSSKLQKLT  
TLVVKVPSLREIEGLEELKSLQDLYLEGCTSLGRL.....PLEKLKE  
LDIGGC (SEQ ID NO:12)

- or having at least 60% similarity thereto or more preferably at least 70% similarity or
- 30 even more preferably at least 80% similarity thereto. Alternatively or in addition to, the rust resistance gene further encodes a p-Loop at its 5' end encoding the amino acid sequence:

GXXXXGKT/S (SEQ ID NO:8),

where X is any amino acid residue. More preferably, the p-Loop sequence comprises the amino acid residues:

GMGGIGKTT (SEQ ID NO:9),

and more particularly

5 GLYGMGGIGKTT (SEQ ID NO:10),

or having one or more amino acid substitutions, insertions and/or deletions thereto provided that such derivatives still function as a p-Loop. A p-Loop is involved in ATP/GTP binding. It is proposed herein that copy number and amino acid sequence of this repeated element are the determinants of the gene-for-gene specificity of rust  
10 resistance genes. Mixing and matching of these elements from existing genes will provide a potential means for creating new and useful resistance gene specificities for use in plant breeding. Such new genes are referred to herein as "modular resistance genes". This is a completely novel approach to disease resistance breeding.

15 According to this embodiment, there is contemplated a modular resistance gene characterised in that said gene encodes a non-naturally occurring leucine rich region. By "non-naturally occurring" is meant to include the manipulation of a genetic sequence to introduce a leucine rich encoding sequence, to increase the copy number of an existing leucine rich encoding sequence, to delete or insert a nucleotide  
20 sequence for or into a leucine rich encoding sequence or to otherwise modify a leucine rich encoding sequence to modulate pathogen specificity of a disease resistance gene. For example, the leucine rich regions of two or more of alleles of the L locus such as L6, L2 and L10 could be combined to broaden the range of pathogens to which a gene can confer resistance. Alternatively, combinations of  
25 alleles from the L and M loci could be made. The present invention contemplates, therefore, a method of producing a modular resistance gene conferring resistance to a pathogen in a plant by manipulating the leucine rich regions in a disease resistance gene.



The present invention further extends to transgenic plants such as transgenic crop plants (e.g. wheat, barley or maize) carrying a non-indigenous genetic sequence conferring or otherwise facilitating rust resistance in said plant. Preferably, the non-indigenous genetic sequence is from a closely related species to the transgenic plant. The expression of the non-indigenous genetic sequence may be constitutive or inducible or developmentally regulated. Furthermore, the non-indigenous sequence may be inserted into or fused to a particular endogenous genetic sequence. In a most preferred embodiment, the transgenic plant is wheat and the non-indigenous genetic sequence is from a wild variety of wheat, barley, maize, flax or *Linum* species. Other transformed species or resistance gene sources are not excluded.

The present invention is further described by reference to the following non-limiting Figures and Examples.

In the Figures:

Figure 1 is a representation of the nucleotide sequence (SEQ ID NO:1) and corresponding amino acid sequence (SEQ ID NOs:2 to 5) of the rust resistance gene L6. There are two predicted products, the "full length" and "truncated" products. The truncated product results from an alternately spliced mRNA that retains intron 3. In the absence of the splicing of this intron, translation would continue through the 82bp intron 3 as a consequence of not changing frames, a stop codon is reached just downstream of the end of the intron. This results in a product from which the major portion of the leucine rich region is removed and also the addition of an extra 29 amino (see underlined amino acids [SEQ ID NOs: 6 and 7) that are not in the full length product.

Figure 2 is a representation of part of the L6 gene showing the LU-1 probe (underlined) and Ac insertion in mutant X75 (SEQ ID NO:11).

Figure 3 is a schematic representation of the L6 gene showing location of LU-2 probe.

- 5 Figure 4 is a representation of the amino acid sequences of the leucine rich repeat in L6.

Figure 5 is a schematic representation of the L locus alleles.

10

#### EXAMPLE 1

##### PLANT MATERIAL

- Experiments were conducted in the line of flax called "Forge" which is homozygous for the rust resistance genes  $L^6$ , M, N and  $P^2$  (2). One particular flax is designated TC257 and is a primary transformant of "Forge" possessing 10 copies of the maize transposable element Ac (1). Rust gene cultivars are referred to as "Birio" ( $L^6$ ),  
15 "Dakota" (M), "Bombay" (N) and "Abyssinian" ( $P^2$ ) (8).

#### EXAMPLE 2

##### RUST STRAINS

- 20 Four rust strains were used to screen for mutants. Three of these strains, designated CH5-78, CH5-84 and CH5-133, were obtained by selfing strain CH5. The fourth strain designated C was a parent strain of CH5. The origins of C and CH5 have been previously described (7). Each of these strains is avirulent on one of the single gene differentials and virulent on the other three. Strain CH5-84 is avirulent on  
25 "Birio" ( $L^6$ ), CH5-78 is avirulent on "Dakota" (M), C is avirulent on "Bombay" (N) and CH5-133 is avirulent on "Abyssinian" ( $P^2$ ). Rust maintenance and inoculation procedures were as previously described (8).

**EXAMPLE 3****TRANSFORMATION**

- Flax cotyledons were transformed with either the vector pKU3 (9), pBT175 (10),  
5 pB135SAc11, 12 (11) or an Ac element in which the OCS enhancer (12) had been  
inserted at the BamH1 site at position 182, according to the protocol previously  
described (13).

**EXAMPLE 4****TAGGING SCHEME**

- The tagging scheme used in this study has been described by Ellis *et al* (1) and  
Lawrence *et al* (13). "Forge" plants with and without Ac elements, including TC257  
and its selfed progeny, were extensively crossed as the female parent with  
"Hoshangabad" to produce hybrid seed heterozygous for the four rust resistance  
15 genes. When seedlings were about 5cm tall, the tip was cut off and the two lateral  
shoots that subsequently developed were inoculated with a mixture of the four rust  
strains, each of which recognise a single resistance gene. Most plants were resistant  
to all four rust strains. Susceptible mutants were of three types, namely, "whole", in  
which all leaves on both lateral shoots were susceptible, "bisected", in which all  
20 leaves on one lateral were susceptible while the other shoot was entirely resistant and  
"mini-sectored", in which only some of the leaves on one shoot were susceptible.  
When rare rust-susceptible mutants were detected, the mutant gene was identified by  
recovering rust spores and inoculating these on to a set of four rust resistance genes  
L<sup>6</sup>, M, N or P<sup>2</sup>. If, for example, the rust recovered from a susceptible mutant plant  
25 grew on the M, N and P<sup>2</sup> differentials but not on the L<sup>6</sup> differential, then this  
indicated that the plant was mutant for the L<sup>6</sup> gene.

**EXAMPLE 5****DETECTION OF TRANSPOSED Ac ELEMENTS IN MUTANT PLANTS**

- 30 The procedure for identifying transposed Ac elements was as previously described  
(2).

**EXAMPLE 6****LAMBDA CLONING**

Flax DNA was digested with *Bam*HI or *Bgl*II and the unfractionated DNA was cloned into the Lambda vector EMBL4 as previously described (3).

5

**EXAMPLE 7****GENETIC ANALYSIS**

Genetic analysis including RFLP techniques, PCR analysis, Southern blot analysis and linkage analysis were as previously described (1; 2). The nucleotide and  
10 corresponding amino acid sequence for the L6 gene is shown in Figure 1. There are two predicted products, the "full length" and "truncated" products. The truncated product results from an alternately spliced mRNA that retains intron 3. In the absence of the splicing of this intron, translation would continue through the 82bp  
15 intron 3 and as a consequence of not changing frames, a stop codon is reached just downstream of the end of the intron. This results in a product from which the major portion of the leucine rich region is removed and also the addition of an extra 29 amino acids (see underlined amino acids in Figure 1) that are not in the full length product.

20

**EXAMPLE 8****IDENTIFICATION AND CHARACTERISATION OF A RUST-SUSCEPTIBLE  
MUTANT PLANT IN WHICH THE L6 GENE HAS BEEN TAGGED BY Ac**

The TC257 (Example 1) line of transgenic flax containing at least 10 copies of Ac  
(1; 2), one of which was linked (29 map units) from the L6 rust resistance gene, was  
25 crossed extensively to a line without resistance genes and the progeny were screened for lack of L6 activity. One rust susceptible mutant lacking L6 specificity (designated mutant X75) contained a transposed Ac element which, from experimental evidence, is putatively located in the L6 gene.

**EXAMPLE 9****X75 CONTAINS A TRANSPOSED AC CLOSELY LINKED TO THE  
MUTANT L6 GENE**

- 5 The L6 mutant X75 contains a transposed Ac (referred to herein as "Tr-Ac") that is not present in its resistant parent. The location of this element was mapped with respect to the L6 gene. A joint segregation analysis of Tr-Ac and the mutant L6 gene demonstrated tight linkage of these two characters. X75 was crossed to cultivar "Birio" (L6) and progeny that inherited Tr-Ac were identified by Southern analysis.
- 10 Two progeny plants, D766 and D769, were test-crossed to the cultivar "Hoshangabad" (no L6). Thirty six progeny were tested for L6 rust resistance and scored for the presence of Tr-Ac. Eighteen of the progeny were susceptible and these all possessed Tr-Ac while the remaining 18 were resistant and these all lacked Tr-Ac. This is the result expected if the allele for susceptibility is an L6 gene inactivated by
- 15 Tr-Ac.

- The insertion site of Tr-Ac was also mapped by restriction fragment length polymorphism (RFLP) analysis in a family of 88 test-cross progeny in which L6 was segregating. A fragment of flax DNA (probe LU-1) isolated from a lambda clone
- 20 containing the 3' junction between Tr-Ac and flax DNA, detected 3 RFLPs that distinguished the two parents when their DNA was cut with *EcoRI*, *BglII* and *XbaI*. The analysis of the segregation of these three markers and the L6 gene in the test-cross demonstrated that all four markers were closely linked. A single recombinant individual involving L6 and the RFLP marker detected in *XbaI* digested DNA was
- 25 detected among the 88 progeny. As detailed below, this recombination event present in progeny plant D237 occurred within the region of the L6 gene.

**EXAMPLE 10****RECOMBINATION IN THE VICINITY OF THE Ac INSERTION SITE  
ALTERS THE RESISTANCE REACTION OR SPECIFICITY OF L6**

- 5 The RFLP probe LU-1 was used to establish a restriction map of the homologous regions of the resistant and susceptible parents of the test cross family and of the recombinant progeny plant D237. Three polymorphic restriction sites were detected and comparison of the maps demonstrated that a cross-over had occurred within a region of about 3kbp on either side of the Ac insertion site.

10

- The recombinant plant D237 was selfed and 16 progeny were screened with a rust isolate that recognises L6. The progeny were also analysed by Southern blotting to detect the recombinant chromosome. The progeny segregated for rust reaction: 6 were fully susceptible while 10 were partially resistant with restricted rust growth confined to the younger leaves. This result is consistent with the segregation of a novel gene for partial resistance which was not present in the original Forge parent. There was a complete association between the second class with novel rust reaction and the presence of the recombinant chromosome.

- 20 Thus, crossing over in the vicinity of the L6 gene results in an alteration of the L6 resistance phenotype.

**EXAMPLE 11****INDEPENDENT L6 MUTANTS CONTAIN INSERTIONS  
IN THE REGION OF 0.5 TO 3.0KB OF THE Ac INSERTION SITE**

- 25 Thirty four independent L6 mutants were isolated in the screen for tagged L6 genes. All but X75 were either deletions or, if not deletions, contained no transposed Ac elements (2). Analysis of mutants in the latter class using the LU-1 probe revealed that two of them (X3A and X117) contained a small (approximately 200bp) insertion in this region. In the case of X117, the mutant was compared to its resistant parent to confirm that the polymorphism was not pre-existing. In the case of X3A, the insertion and loss of L6 had occurred somatically. Mutant X3A arose as a sector. Among two shoots on a single F1 plant, one (X3A) had lost L6 and the second, X3B was wild-type for L6. DNA from the two sectors was examined using the LU-1 probe. An insertion was observed only in DNA from the mutant sector.
- 30
- 35

**EXAMPLE 12****EXCISION OF TR-AC IN PROGENY OF X75 IS ASSOCIATED  
WITH REVERSION TO RUST RESISTANCE**

The X75 mutant was selfed and progeny that were homozygous for the transposed Ac element were identified by Southern blotting. Selfed progeny of four such plants were in turn screened with a rust strain that recognises the L6 resistance specificity. Thirty seven resistant plants were identified among 3105 progeny. 15 of the revertants were examined by either PCR or Southern analysis for an excision fragment that would result from the excision of Ac from the L6 region. The excision marker occurred in all the plants. In two cases, the excision region was sequenced and small base changes, (an "Ac footprint") in the 8bp repeat that flanked Ac in X75 resulted from the excision process.

**EXAMPLE 13****A DNA PROBE ADJACENT TO Ac DETECTS A SECOND LOCUS  
IN FLAX, CLOSELY LINKED TO THE M RESISTANCE LOCUS**

A second DNA probe, LU-2 was isolated from the same lambda clone as LU-1. These two fragments are separated by about 2kb. LU-2, like many other flax DNA probes, hybridises to two genomic fragments in most restriction digests, providing molecular evidence for the tetraploid nature of flax. In *Xba*I digests, LU-2 detects two polymorphic markers, LU-2-1 and LU-2-2 that distinguish the resistant and susceptible parents of a test-cross family of 52 progeny among which the L6 and M resistance genes segregate. Joint segregation analysis of these two RFLP markers and the L6 and M resistance genes demonstrated complete linkage of LU-2-1 with L6 and LU-2-2 with the resistance gene M. Thus, cloning the L6 locus has allowed access to the M resistance gene region. The LU-2 probe and A flanked Ac in X75 in L6 is shown in Figures 2 and 3.

**EXAMPLE 14****RUST RESISTANCE GENES FORM A MULTIGENE FAMILY IN FLAX**

The probe LU-1 and several adjacent restriction fragments from a lambda clone were used as hybridisation probes to isolate a cDNA from a library generated using leaf mRNA. A cDNA clone and several other probes from the L6 gene have been used as probes for genomic Southern analysis. These probes generally detect multiple fragments in Southern blots which indicates that flax contains a family of genes of similar sequence. All RFLPs detected with this probe map to either the L6 or M resistance gene regions.

**EXAMPLE 15****PROBE LU-1 IDENTIFIES ALLELES OF L6**

In genetic studies, the L locus of flax behaves as a single gene with multiple allelic specificities. Twelve resistance specificities map to the L locus. The probe LU-1 was used to examine a differential set of flax plants that each contain a single L allele (see Figures 2 and 3). DNA from the 12 differentials and from "Hoshangabad" which carries a "null" allele at the L locus was cut with *Xba*I, *Bgl*II and *Eco*RI. In each case, a single fragment was detected in the L differentials and each L allele could be distinguished from the null allele of "Hoshangabad". Using these three enzymes, 10 RFLP genotypes were detected. Only two pairs of L alleles, L3 and L4 and L6 and L7, could not be distinguished with this limited sample of digests.

**EXAMPLE 16****A cDNA FROM FLAX DETECTS SEQUENCE SIMILARITY IN****A WILD LINUM SPECIES**

Southern analysis of DNA from the wild species, *Linum marginale* which contains genes controlling resistance against flax rust, detected strongly hybridising fragments.

**EXAMPLE 17****DETERMINATION OF AMINO ACID SEQUENCE OF L6**

The amino acid sequence of the L6 cDNA was determined and is shown in Figure 1. The amino acid sequence is shown in single letter code, single letter abbreviations for amino acids are defined below:



	<u>Amino Acid</u>	<u>One-letter Symbol</u>
	Alanine	A
5	Arginine	R
	Asparagine	N
	Aspartic acid	D
	Cysteine	C
	Glutamine	Q
10	Glutamic acid	E
	Glycine	G
	Histidine	H
	Isoleucine	I
	Leucine	L
15	Lysine	K
	Methionine	M
	Phenylalanine	F
	Proline	P
	Serine	S
20	Threonine	T
	Tryptophan	W
	Tyrosine	Y
	Valine	V
	Amino Acid	X

**EXAMPLE 18**  
COMPARISON OF AMINO ACID SEQUENCE OF  
LEUCINE RICH REPEAT FROM L6

5

The L6 allele comprises a leucine rich repeat at amino acid residues 969 to 1115 and 1116 to 1265. This repeat was compared for identity and the comparison is shown in Figure 4. Details of the comparisons are shown below:

10	Length:	156 amino acids
	Gaps:	3
	Gap weight:	3.000
	Length weight:	0.100
	Quality:	160.4
15	Ratio:	1.091
	Average match:	0.540
	Average mismatch:	-0.396
	Percent similarity:	82.979%
	Percent identity:	74.468

20

**EXAMPLE 19**  
CLONING AND COMPARISON OF L LOCUS ALLELES

Using the clones L6 gene as a probe (probe LU-1), the L2 and L10 alleles were  
 25 cloned on *EcoRI* DNA fragments. These alleles have different resistance specificities and control resistance to flax rust strains that carry the AL2 and AL10 avirulence genes, respectively.

The L2 and L10 clones were subject to restriction endonuclease analysis and a  
 30 schematic represented is shown in Figure 5. The L2 *EcoRI* fragment is about 400bp longer than the corresponding L10 fragment. DNA sequence analysis of both ends of the L2 and L10 fragments revealed practically identical sequence (>90%) and indicates that L2 differs from L10 by about 400bp of extra DNA. Furthermore, sequence analysis and restriction fragment mapping from internal restriction sites  
 35 confirmed the extra DNA and indicated that the 5' halves of L2, L6 and L10 are very similar (>90% identical) and that the differences between the alleles occurs in the 3' half of the gene. The difference in gene length occurs in a region coding for the leucine rich repeat structure of 156 amino acids that has two copies in L6 (Figure 4)

and only one copy in a cDNA from a related but non-allelic gene called FC4.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It

- 5 is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: COMMONWEALTH SCIENTIFIC AND  
INDUSTRIAL RESEARCH ORGANISATION
- (ii) TITLE OF INVENTION: GENETIC SEQUENCES CONFERRING  
DISEASE RESISTANCE IN PLANTS AND  
USES THEREFOR
- (iii) NUMBER OF SEQUENCES: 13
- (iv) CORRESPONDENCE ADDRESS:
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  - (E) COUNTRY: AUSTRALIA
  - (F) ZIP: 3000
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
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- 28 -

## (2) INFORMATION FOR SEQ ID NO:1:

- (1) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 4841 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(11) MOLECULE TYPE: DNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 163..789

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1097..2203

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2669..4525

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2293..2586

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GAGCTCAGAA GTAAGGGATA AGGCAAGAAG CAGAGGAGCA GAGAGAAGAA CCAGCAAAGC      60
ACATGCAAAT TGAAGCAGGC AAGAACAGTT ACTGGAAATT CATTTATCTC TGCTTTCAAT      120
TTCTATCCTT CAGATCATTT CTGCTCAATT GAATCACTAG TC ATG AGT TAT TTG          174
                                     Met Ser Tyr Leu
                                     1

AGA GAA GTT GCT ACT GCT GTT GCC TTG CTT CTC CCT TTC ATT CTT CTC          222
Arg Glu Val Ala Thr Ala Val Ala Leu Leu Leu Pro Phe Ile Leu Leu
   5              10              15              20

AAC AAG TTT TGG AGA CCA AAT TCC AAA GAC TCA ATC GTC AAC GAT GAT          270
Asn Lys Phe Trp Arg Pro Asn Ser Lys Asp Ser Ile Val Asn Asp Asp
          25              30              35

GAC GAT TCA ACA TCT GAA GTT GAT GCC ATA TCC GAC TCC ACA AAT CCC          318
Asp Asp Ser Thr Ser Glu Val Asp Ala Ile Ser Asp Ser Thr Asn Pro
          40              45              50

TCT GGT TCA TTT CCC TCC GTG GAG TAT GAA GTG TTT TTG AGT TTC AGG          366
Ser Gly Ser Phe Pro Ser Val Glu Tyr Glu Val Phe Leu Ser Phe Arg
          55              60              65

GGT CCA GAT ACT CGT GAA CAG TTC ACC GAT TTC CTA TAT CAG TCT CTC          414
Gly Pro Asp Thr Arg Glu Gln Phe Thr Asp Phe Leu Tyr Gln Ser Leu
          70              75              80

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CGT CGC TAT AAG ATT CAC ACT TTT AGG GAC GAC GAT GAG CTA CTC AAA	462
Arg Arg Tyr Lys Ile His Thr Phe Arg Asp Asp Asp Glu Leu Leu Lys	
85 90 95 100	
GGA AAA GAA ATA GGG CCC AAC CTC CTA CGA GCA ATT GAT CAG TCC AAA	510
Gly Lys Glu Ile Gly Pro Asn Leu Leu Arg Ala Ile Asp Gln Ser Lys	
105 110 115	
ATT TAC GTC CCG ATC ATA TCG AGC GGA TAT GCT GAT AGT AAG TGG TGT	558
Ile Tyr Val Pro Ile Ile Ser Ser Gly Tyr Ala Asp Ser Lys Trp Cys	
120 125 130	
CTT ATG GAG CTT GCT GAA ATT GTG AGA CGT CAA GAG GAG GAC CCT CGA	606
Leu Met Glu Leu Ala Glu Ile Val Arg Arg Gln Glu Glu Asp Pro Arg	
135 140 145	
CGC ATC ATA CTT CCT ATT TTT TAT ATG GTG GAT CCA AGT GAC GTA CGA	654
Arg Ile Ile Leu Pro Ile Phe Tyr Met Val Asp Pro Ser Asp Val Arg	
150 155 160	
CAT CAG ACT GGA TGT TAT AAA AAA GCA TTT CGA AAA CAC GCA AAT AAA	702
His Gln Thr Gly Cys Tyr Lys Lys Ala Phe Arg Lys His Ala Asn Lys	
165 170 175 180	
TTT GAT GGA CAG ACC ATA CAA AAC TGG AAA GAT GCT CTA AAG AAG GTC	750
Phe Asp Gly Gln Thr Ile Gln Asn Trp Lys Asp Ala Leu Lys Lys Val	
185 190 195	
GGA GAC TTA AAA GGA TGG CAC ATC GGA AAG AAT GAC AAG TATGTAATCC	799
Gly Asp Leu Lys Gly Trp His Ile Gly Lys Asn Asp Lys	
200 205	
TCATCCTAGC CTTTATCAT TCAGTACAAC TTATTGTTT TGTCTGCAAT ATACTTACTT	859
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AAGTTAAAAT GCTCAATAAC GTGGAATATA TACTTGTATA CAGGGGCAAC AAACATATAA	1039
TTGAAATATT ATTTCATATG AAGTTAACAT CTCAAAAATG TATTTAATAC TTGTAGG	1096
CAG GGA GCT ATA GCA GAC AAA GTA TCA GCA GAT ATA TGG TCA CAC ATA	1144
Gln Gly Ala Ile Ala Asp Lys Val Ser Ala Asp Ile Trp Ser His Ile	
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AGC AAG GAA AAT CTC ATT TTA GAA ACC GAT GAG TTG GTT GGA ATT GAT	1192
Ser Lys Glu Asn Leu Ile Leu Glu Thr Asp Glu Leu Val Gly Ile Asp	
20 25 30	
GAT CAC ATA ACA GCC GTA TTA GAA AAA TTG AGT TTA GAC TCT GAA AAT	1240
Asp His Ile Thr Ala Val Leu Glu Lys Leu Ser Leu Asp Ser Glu Asn	
35 40 45	

- 30 -

GTG ACA ATG GTC GGC CTT TAT GGT ATG GGT GGA ATA GGC AAG ACG ACC	1288
Val Thr Met Val Gly Leu Tyr Gly Met Gly Gly Ile Gly Lys Thr Thr	
50 55 60	
ACT GCA AAG GCG GTT TAT AAC AAG ATT TCT TCT TGT TTC GAT TGT TGT	1336
Thr Ala Lys Ala Val Tyr Asn Lys Ile Ser Ser Cys Phe Asp Cys Cys	
65 70 75 80	
TGT TTT ATT GAC AAC ATA CGT GAA ACA CAA GAG AAG GAT GGC GTT GTT	1384
Cys Phe Ile Asp Asn Ile Arg Glu Thr Gln Glu Lys Asp Gly Val Val	
85 90 95	
GTT TTG CAA AAG AAG CTA GTA TCT GAA ATT TTG AGG ATC GAT TCG GGT	1432
Val Leu Gln Lys Lys Leu Val Ser Glu Ile Leu Arg Ile Asp Ser Gly	
100 105 110	
TCG GTT GGA TTT AAT AAT GAT AGT GGC GGA CGG AAG ACG ATA AAG GAG	1480
Ser Val Gly Phe Asn Asn Asp Ser Gly Gly Arg Lys Thr Ile Lys Glu	
115 120 125	
AGA GTT TCG AGG TTC AAA ATT CTT GTC GTT CTC GAT GAT GTG GAT GAG	1528
Arg Val Ser Arg Phe Lys Ile Leu Val Val Leu Asp Asp Val Asp Glu	
130 135 140	
AAG TTT AAA TTT GAA GAT ATG TTG GGA AGT CCT AAA GAT TTT ATT TCT	1576
Lys Phe Lys Phe Glu Asp Met Leu Gly Ser Pro Lys Asp Phe Ile Ser	
145 150 155 160	
CAA AGT AGA TTC ATT ATT ACT TCA AGA AGT ATG AGA GTT TTG GGT ACT	1624
Gln Ser Arg Phe Ile Ile Thr Ser Arg Ser Met Arg Val Leu Gly Thr	
165 170 175	
TTG AAT GAG AAT CAA TGC AAG TTG TAT GAA GTT GGA TCG ATG AGC AAA	1672
Leu Asn Glu Asn Gln Cys Lys Leu Tyr Glu Val Gly Ser Met Ser Lys	
180 185 190	
CCA CGT TCG CTT GAA CTC TTC TCC AAG CAT GCA TTC AAA AAG AAT ACG	1720
Pro Arg Ser Leu Glu Leu Phe Ser Lys His Ala Phe Lys Lys Asn Thr	
195 200 205	
CCT CCA TCG TAT TAT GAG ACT CTA GCA AAT GAC GTC GTA GAT ACT ACA	1768
Pro Pro Ser Tyr Tyr Glu Thr Leu Ala Asn Asp Val Val Asp Thr Thr	
210 215 220	
GCA GGA CTT CCA TTG ACT CTG AAG GTT ATA GGA TCG CTT TTA TTT AAA	1816
Ala Gly Leu Pro Leu Thr Leu Lys Val Ile Gly Ser Leu Leu Phe Lys	
225 230 235 240	
CAA GAG ATT GCG GTT TGG GAA GAC ACG TTG GAA CAA TTA CGT AGA ACA	1864
Gln Glu Ile Ala Val Trp Glu Asp Thr Leu Glu Gln Leu Arg Arg Thr	
245 250 255	
CTT AAC CTT GAT GAG GTT TAT GAT AGG CTA AAA ATA AGT TAT GAT GCG	1912
Leu Asn Leu Asp Glu Val Tyr Asp Arg Leu Lys Ile Ser Tyr Asp Ala	
260 265 270	



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TTG AAC CCG GAG GCA AAA GAG ATT TTC TTG GAT ATA GCT TGC TTC TTC Leu Asn Pro Glu Ala Lys Glu Ile Phe Leu Asp Ile Ala Cys Phe Phe 275 280 285	1960
ATC GGA CAA AAT AAA GAA GAA CCG TAT TAC ATG TGG ACC GAC TGT AAT Ile Gly Gln Asn Lys Glu Glu Pro Tyr Tyr Met Trp Thr Asp Cys Asn 290 295 300	2008
TTT TAT CCA GCA AGT AAT ATT ATT TTT CTC ATT CAA AGA TGT ATG ATA Phe Tyr Pro Ala Ser Asn Ile Ile Phe Leu Ile Gln Arg Cys Met Ile 305 310 315 320	2056
CAA GTT GGG GAT GAT GAT GAG TTT AAA ATG CAC GAC CAA CTT AGA GAT Gln Val Gly Asp Asp Asp Glu Phe Lys Met His Asp Gln Leu Arg Asp 325 330 335	2104
ATG GGT AGA GAA ATT GTG AGA CGA GAG GAT GTA CTG CCG TGG AAG AGA Met Gly Arg Glu Ile Val Arg Arg Glu Asp Val Leu Pro Trp Lys Arg 340 345 350	2152
AGT AGA ATA TGG TCG GCA GAA GAA GGG ATT GAT CTC TTG CTG AAC AAA Ser Arg Ile Trp Ser Ala Glu Glu Gly Ile Asp Leu Leu Leu Asn Lys 355 360 365	2200
AAG GTATTCAGTT TTATTAAAA TTAAATATTC ATATATTATT ACACATACTT Lys	2253
TAAATCACAT AACTCATTAC GTTCCTTCTC CAATTACAG GGA TCA AGT AAA GTA Gly Ser Ser Lys Val 1 5	2307
AAA GCA ATT AGC ATA CCC TGG GGT GTC AAG TAT GAG TTT AAG AGC GAA Lys Ala Ile Ser Ile Pro Trp Gly Val Lys Tyr Glu Phe Lys Ser Glu 10 15 20	2355
TGT TTC TTG AAT TTG TCA GAG TTG AGA TAC CTC CAT GCA AGG GAA GCC Cys Phe Leu Asn Leu Ser Glu Leu Arg Tyr Leu His Ala Arg Glu Ala 25 30 35	2403
ATG CTT ACC GGA GAT TTC AAC AAT CTT CTC CCG AAT TTA AAG TGG CTT Met Leu Thr Gly Asp Phe Asn Asn Leu Leu Pro Asn Leu Lys Trp Leu 40 45 50	2451
GAG TTG CCA TTT TAC AAA CAT GGA GAG GAT GAT CCT CCT TTG ACC AAT Glu Leu Pro Phe Tyr Lys His Gly Glu Asp Asp Pro Pro Leu Thr Asn 55 60 65	2499
TAT ACC ATG AAA AAT CTG ATA ATT GTT ATT CTT GAG CAT AGC CAC ATA Tyr Thr Met Lys Asn Leu Ile Ile Val Ile Leu Glu His Ser His Ile 70 75 80 85	2547
ACG GCT GAT GAT TGG GGA GGT TGG AGG CAT ATG ATG AAG GTGTGTTGTT Thr Ala Asp Asp Trp Gly Gly Trp Arg His Met Met Lys 90 95	2596

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TTTCAGCTGT TCATATGAAG GTGTGTATC TTCTTATTG TTCTTCATAT TTCTGTTTTA	2656
ATCTGCTGTC AG ATG GCT GAG AGG CTG AAA GTT GTA CGA CTT GCT TCA	2704
Met Ala Glu Arg Leu Lys Val Val Arg Leu Ala Ser	
1 5 10	
AAC TAT AGT TTG TAC GGA AGA CGT GTT CGC CTT TCT GAC TGT TGG CGC	2752
Asn Tyr Ser Leu Tyr Gly Arg Arg Val Arg Leu Ser Asp Cys Trp Arg	
15 20 25	
TTC CCC AAA AGC ATT GAG GTA TTA TCC ATG ACT GCG ATA GAA ATG GAT	2800
Phe Pro Lys Ser Ile Glu Val Leu Ser Met Thr Ala Ile Glu Met Asp	
30 35 40	
GAA GTT GAT ATT GGG GAG TTA AAG AAG CTA AAG ACG TTG GTT CTG AAA	2848
Glu Val Asp Ile Gly Glu Leu Lys Lys Leu Lys Thr Leu Val Leu Lys	
45 50 55 60	
TTC TGT CCA ATA CAA AAG ATA AGT GGG GGA ACC TTT GGT ATG TTG AAG	2896
Phe Cys Pro Ile Gln Lys Ile Ser Gly Gly Thr Phe Gly Met Leu Lys	
65 70 75	
GGA CTT CGA GAG CTT TGT CTC GAA TTC AAC TGG GGG ACA AAT TTG AGA	2944
Gly Leu Arg Glu Leu Cys Leu Glu Phe Asn Trp Gly Thr Asn Leu Arg	
80 85 90	
GAG GTA GTT GCC GAT ATT GGT CAA CTT TCA TCT CTC AAA GTC TTG AAA	2992
Glu Val Val Ala Asp Ile Gly Gln Leu Ser Ser Leu Lys Val Leu Lys	
95 100 105	
ACA ACC GGA GCT AAG GAG GTC GAG ATT AAT GAA TTT CCA TTA GGT TTG	3040
Thr Thr Gly Ala Lys Glu Val Glu Ile Asn Glu Phe Pro Leu Gly Leu	
110 115 120	
AAG GAG TTA TCC ACT TCA TCT CGG ATT CCG AAT CTT TCA CAG TTG TTG	3088
Lys Glu Leu Ser Thr Ser Ser Arg Ile Pro Asn Leu Ser Gln Leu Leu	
125 130 135 140	
GAT TTG GAG GTA CTG AAG GTT TAT GAT TGC AAG GAT GGA TTT GAC ATG	3136
Asp Leu Glu Val Leu Lys Val Tyr Asp Cys Lys Asp Gly Phe Asp Met	
145 150 155	
CCT CCT GCT AGT CCG AGT GAA GAT GAA AGT AGT GTG TGG TGG AAG GTA	3184
Pro Pro Ala Ser Pro Ser Glu Asp Glu Ser Ser Val Trp Trp Lys Val	
160 165 170	
TCC AAG TTG AAG TCT TTG CAA CTC GAG AAG ACA AGA ATC AAT GTC AAC	3232
Ser Lys Leu Lys Ser Leu Gln Leu Glu Lys Thr Arg Ile Asn Val Asn	
175 180 185	
GTT GTG GAT GAT GCT TCT TCC GGT GGT CAC CTC CCT CGT TAC TTA CTA	3280
Val Val Asp Asp Ala Ser Ser Gly Gly His Leu Pro Arg Tyr Leu Leu	
190 195 200	

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CCA ACA TCC CTA ACC TAT CTT AAA ATT TAT CAG TGT ACA GAA CCA ACG Pro Thr Ser Leu Thr Tyr Leu Lys Ile Tyr Gln Cys Thr Glu Pro Thr 205 210 215 220	3328
TGG CTT CCA GGA ATA GAG AAC TTG GAG AAT TTG ACT TCG CTG GAA GTC Trp Leu Pro Gly Ile Glu Asn Leu Glu Asn Leu Thr Ser Leu Glu Val 225 230 235	3376
AAC GAC ATC TTC CAA ACT CTT GGA GGT GAC TTG GAT GGG CTA CAA GGG Asn Asp Ile Phe Gln Thr Leu Gly Gly Asp Leu Asp Gly Leu Gln Gly 240 245 250	3424
TTG AGA TCA TTG GAA ATT CTT AGG ATT CGG AAA GTA AAT GGT TTA GCT Leu Arg Ser Leu Glu Ile Leu Arg Ile Arg Lys Val Asn Gly Leu Ala 255 260 265	3472
CGG ATC AAA GGG CTT AAG GAT CTC TTG TGT TCT TCT ACC TGC AAG TTG Arg Ile Lys Gly Leu Lys Asp Leu Leu Cys Ser Ser Thr Cys Lys Leu 270 275 280	3520
CGG AAA TTT TAT ATT ACA GAA TGC CCC GAC CTC ATT GAG TTA CTC CCA Arg Lys Phe Tyr Ile Thr Glu Cys Pro Asp Leu Ile Glu Leu Leu Pro 285 290 295 300	3568
TGC GAA CTC GGC GGC CAA ACA GTA GTA GTC CCC TCT ATG GCA GAA CTG Cys Glu Leu Gly Gly Gln Thr Val Val Val Pro Ser Met Ala Glu Leu 305 310 315	3616
ACC ATT AGG GAT TGT CCA CGG CTG GAG GTT GGC CCG ATG ATA AGA TCA Thr Ile Arg Asp Cys Pro Arg Leu Glu Val Gly Pro Met Ile Arg Ser 320 325 330	3664
CTC CCA AAG TTC CCA ATG CTA AAG AAG TTG GAC CTC GCG GTG GCA AAT Leu Pro Lys Phe Pro Met Leu Lys Lys Leu Asp Leu Ala Val Ala Asn 335 340 345	3712
ATA ACT AAA GAG GAG GAT CTG GAT GCG ATT GGA TCC CTA GAA GAG TTG Ile Thr Lys Glu Glu Asp Leu Asp Ala Ile Gly Ser Leu Glu Glu Leu 350 355 360	3760
GTT AGT TTG GAG TTA GAG TTA GAC GAT ACA TCT TCC GGT ATA GAG AGG Val Ser Leu Glu Leu Glu Leu Asp Asp Thr Ser Ser Gly Ile Glu Arg 365 370 375 380	3808
ATA GTT TCC TCT TCG AAG CTG CAA AAG TTA ACT ACA CTC GTA GTG AAG Ile Val Ser Ser Ser Lys Leu Gln Lys Leu Thr Thr Leu Val Val Lys 385 390 395	3856
GTG CCG AGT TTG CGG GAG ATT GAA GGG CTT GAA GAG TTG AAG TCT TTA Val Pro Ser Leu Arg Glu Ile Glu Gly Leu Glu Glu Leu Lys Ser Leu 400 405 410	3904
CAA GAT TTG TAT CTA GAG GGT TGC ACG TCG TTG GGG AGA CTA CCA CTG Gln Asp Leu Tyr Leu Glu Gly Cys Thr Ser Leu Gly Arg Leu Pro Leu 415 420 425	3952

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GAG AAG CTG AAG GAG CTA GAC ATT GGA GGA TGC CCT GAC CTC ACT GAG Glu Lys Leu Lys Glu Leu Asp Ile Gly Gly Cys Pro Asp Leu Thr Glu 430 435 440	4000
TTA GTC CAA ACA GTA GTA GCA GTC CCC TCT TTG AGA GGA CTG ACC ATT Leu Val Gln Thr Val Val Ala Val Pro Ser Leu Arg Gly Leu Thr Ile 445 450 455 460	4048
AGG GAT TGT CCA CGG CTG GAG GTT GGT CCA ATG ATA CAA TCT CTT CCA Arg Asp Cys Pro Arg Leu Glu Val Gly Pro Met Ile Gln Ser Leu Pro 465 470 475	4096
AAG TTC CCA ATG CTA AAT GAA TTG ACG CTC TCG ATG GTA AAT ATC ACT Lys Phe Pro Met Leu Asn Glu Leu Thr Leu Ser Met Val Asn Ile Thr 480 485 490	4144
AAG GAG GAT GAG CTG GAG GTG CTT GGA TCC CTA GAA GAG TTG GAT AGT Lys Glu Asp Glu Leu Glu Val Leu Gly Ser Leu Glu Glu Leu Asp Ser 495 500 505	4192
TTG GAG TTA ACG TTA GAC GAT ACA TGT TCC AGC ATA GAG AGG ATA TCT Leu Glu Leu Thr Leu Asp Asp Thr Cys Ser Ser Ile Glu Arg Ile Ser 510 515 520	4240
TTC TTG TCG AAG CTG CAA AAG TTA ACT ACA CTC ATA GTG GAG GTG CCG Phe Leu Ser Lys Leu Gln Lys Leu Thr Thr Leu Ile Val Glu Val Pro 525 530 535 540	4288
AGT TTG CGG GAG ATT GAA GGT CTT GCA GAG TTG AAG TCT TTA CGA ATT Ser Leu Arg Glu Ile Glu Gly Leu Ala Glu Leu Lys Ser Leu Arg Ile 545 550 555	4336
TTG TAT CTA GAA GGA TGC ACG TCG TTG GAA AGA CTG TGG CCT GAT CAA Leu Tyr Leu Glu Gly Cys Thr Ser Leu Glu Arg Leu Trp Pro Asp Gln 560 565 570	4384
CAA CAG TTG GGT AGT CTG AAG AAC CTG AAT GTG CTC GAC ATC CAA GGT Gln Gln Leu Gly Ser Leu Lys Asn Leu Asn Val Leu Asp Ile Gln Gly 575 580 585	4432
TGT AAA AGC TTG AGT GTT GAC CAT CTC TCT GCA CTC AAG ACC ACT CTA Cys Lys Ser Leu Ser Val Asp His Leu Ser Ala Leu Lys Thr Thr Leu 590 595 600	4480
CCG CCC AGG GCG AGG ATA ACA TGG CCC GAT CAG CCC TAC AGA TGACGGTAGG Pro Pro Arg Ala Arg Ile Thr Trp Pro Asp Gln Pro Tyr Arg 605 610 615	4532
AATTAATGCA AGTGACAGTG ATGACATAGT TGTGATGGCT TGCACCTGAT CAACCTTGTT	4592
CTTCATGGGG TTTTCTCCCC AGTGAGATCT TAATATCCAA AATTCTGGTT TGTTCAAGAGG	4652
TTATATGGTT CAGTTTTTCA CCATAATATT TTCTACGGCA TAGCGCAAAC TACTTCTAGT	4712
ATATAGATAT AGGCAATATA TATAACATCC AACTCTGTTT TACTCACTCC TCTCATCTTC	4772

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TCACGCGATT ATGTTCCATT TCTAAAATCC ATTATTCATC GCCTTATATT CATAATTATG 4832  
 TAATTATT 4841

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 208 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ser Tyr Leu Arg Glu Val Ala Thr Ala Val Ala Leu Leu Leu Pro  
 1 5 10 15  
 Phe Ile Leu Leu Asn Lys Phe Trp Arg Pro Asn Ser Lys Asp Ser Ile  
 20 25 30  
 Val Asn Asp Asp Asp Asp Ser Thr Ser Glu Val Asp Ala Ile Ser Asp  
 35 40 45  
 Ser Thr Asn Pro Ser Gly Ser Phe Pro Ser Val Glu Tyr Glu Val Phe  
 50 55 60  
 Leu Ser Phe Arg Gly Pro Asp Thr Arg Glu Gln Phe Thr Asp Phe Leu  
 65 70 75 80  
 Tyr Gln Ser Leu Arg Arg Tyr Lys Ile His Thr Phe Arg Asp Asp Asp  
 85 90 95  
 Glu Leu Leu Lys Gly Lys Glu Ile Gly Pro Asn Leu Leu Arg Ala Ile  
 100 105 110  
 Asp Gln Ser Lys Ile Tyr Val Pro Ile Ile Ser Ser Gly Tyr Ala Asp  
 115 120 125  
 Ser Lys Trp Cys Leu Met Glu Leu Ala Glu Ile Val Arg Arg Gln Glu  
 130 135 140  
 Glu Asp Pro Arg Arg Ile Ile Leu Pro Ile Phe Tyr Met Val Asp Pro  
 145 150 155 160  
 Ser Asp Val Arg His Gln Thr Gly Cys Tyr Lys Lys Ala Phe Arg Lys  
 165 170 175  
 His Ala Asn Lys Phe Asp Gly Gln Thr Ile Gln Asn Trp Lys Asp Ala  
 180 185 190

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Leu Lys Lys Val Gly Asp Leu Lys Gly Trp His Ile Gly Lys Asn Asp  
 195 200 205

Lys

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 369 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Gln Gly Ala Ile Ala Asp Lys Val Ser Ala Asp Ile Trp Ser His Ile  
 1 5 10 15  
 Ser Lys Glu Asn Leu Ile Leu Glu Thr Asp Glu Leu Val Gly Ile Asp  
 20 25 30  
 Asp His Ile Thr Ala Val Leu Glu Lys Leu Ser Leu Asp Ser Glu Asn  
 35 40 45  
 Val Thr Met Val Gly Leu Tyr Gly Met Gly Gly Ile Gly Lys Thr Thr  
 50 55 60  
 Thr Ala Lys Ala Val Tyr Asn Lys Ile Ser Ser Cys Phe Asp Cys Cys  
 65 70 75 80  
 Cys Phe Ile Asp Asn Ile Arg Glu Thr Gln Glu Lys Asp Gly Val Val  
 85 90 95  
 Val Leu Gln Lys Lys Leu Val Ser Glu Ile Leu Arg Ile Asp Ser Gly  
 100 105 110  
 Ser Val Gly Phe Asn Asn Asp Ser Gly Gly Arg Lys Thr Ile Lys Glu  
 115 120 125  
 Arg Val Ser Arg Phe Lys Ile Leu Val Val Leu Asp Asp Val Asp Glu  
 130 135 140  
 Lys Phe Lys Phe Glu Asp Met Leu Gly Ser Pro Lys Asp Phe Ile Ser  
 145 150 155 160  
 Gln Ser Arg Phe Ile Ile Thr Ser Arg Ser Met Arg Val Leu Gly Thr  
 165 170 175  
 Leu Asn Glu Asn Gln Cys Lys Leu Tyr Glu Val Gly Ser Met Ser Lys  
 180 185 190

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Pro Arg Ser Leu Glu Leu Phe Ser Lys His Ala Phe Lys Lys Asn Thr  
 195 200 205

Pro Pro Ser Tyr Tyr Glu Thr Leu Ala Asn Asp Val Val Asp Thr Thr  
 210 215 220

Ala Gly Leu Pro Leu Thr Leu Lys Val Ile Gly Ser Leu Leu Phe Lys  
 225 230 235 240

Gln Glu Ile Ala Val Trp Glu Asp Thr Leu Glu Gln Leu Arg Arg Thr  
 245 250 255

Leu Asn Leu Asp Glu Val Tyr Asp Arg Leu Lys Ile Ser Tyr Asp Ala  
 260 265 270

Leu Asn Pro Glu Ala Lys Glu Ile Phe Leu Asp Ile Ala Cys Phe Phe  
 275 280 285

Ile Gly Gln Asn Lys Glu Glu Pro Tyr Tyr Met Trp Thr Asp Cys Asn  
 290 295 300

Phe Tyr Pro Ala Ser Asn Ile Ile Phe Leu Ile Gln Arg Cys Met Ile  
 305 310 315 320

Gln Val Gly Asp Asp Asp Glu Phe Lys Met His Asp Gln Leu Arg Asp  
 325 330 335

Met Gly Arg Glu Ile Val Arg Arg Glu Asp Val Leu Pro Trp Lys Arg  
 340 345 350

Ser Arg Ile Trp Ser Ala Glu Glu Gly Ile Asp Leu Leu Leu Asn Lys  
 355 360 365

Lys

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 98 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Gly Ser Ser Lys Val Lys Ala Ile Ser Ile Pro Trp Gly Val Lys Tyr  
 1 5 10 15

Glu Phe Lys Ser Glu Cys Phe Leu Asn Leu Ser Glu Leu Arg Tyr Leu  
 20 25 30

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His Ala Arg Glu Ala Met Leu Thr Gly Asp Phe Asn Asn Leu Leu Pro  
 35 40 45  
 Asn Leu Lys Trp Leu Glu Leu Pro Phe Tyr Lys His Gly Glu Asp Asp  
 50 55 60  
 Pro Pro Leu Thr Asn Tyr Thr Met Lys Asn Leu Ile Ile Val Ile Leu  
 65 70 75 80  
 Glu His Ser His Ile Thr Ala Asp Asp Trp Gly Gly Trp Arg His Met  
 85 90 95  
 Met Lys

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 619 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Ala Glu Arg Leu Lys Val Val Arg Leu Ala Ser Asn Tyr Ser Leu  
 1 5 10 15  
 Tyr Gly Arg Arg Val Arg Leu Ser Asp Cys Trp Arg Phe Pro Lys Ser  
 20 25 30  
 Ile Glu Val Leu Ser Met Thr Ala Ile Glu Met Asp Glu Val Asp Ile  
 35 40 45  
 Gly Glu Leu Lys Lys Leu Lys Thr Leu Val Leu Lys Phe Cys Pro Ile  
 50 55 60  
 Gln Lys Ile Ser Gly Gly Thr Phe Gly Met Leu Lys Gly Leu Arg Glu  
 65 70 75 80  
 Leu Cys Leu Glu Phe Asn Trp Gly Thr Asn Leu Arg Glu Val Val Ala  
 85 90 95  
 Asp Ile Gly Gln Leu Ser Ser Leu Lys Val Leu Lys Thr Thr Gly Ala  
 100 105 110  
 Lys Glu Val Glu Ile Asn Glu Phe Pro Leu Gly Leu Lys Glu Leu Ser  
 115 120 125  
 Thr Ser Ser Arg Ile Pro Asn Leu Ser Gln Leu Leu Asp Leu Glu Val  
 130 135 140  
 Leu Lys Val Tyr Asp Cys Lys Asp Gly Phe Asp Met Pro Pro Ala Ser  
 145 150 155 160



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Pro Ser Glu Asp Glu Ser Ser Val Trp Trp Lys Val Ser Lys Leu Lys  
 165 170 175

Ser Leu Gln Leu Glu Lys Thr Arg Ile Asn Val Asn Val Val Asp Asp  
 180 185 190

Ala Ser Ser Gly Gly His Leu Pro Arg Tyr Leu Leu Pro Thr Ser Leu  
 195 200 205

Thr Tyr Leu Lys Ile Tyr Gln Cys Thr Glu Pro Thr Trp Leu Pro Gly  
 210 215 220

Ile Glu Asn Leu Glu Asn Leu Thr Ser Leu Glu Val Asn Asp Ile Phe  
 225 230 235 240

Gln Thr Leu Gly Gly Asp Leu Asp Gly Leu Gln Gly Leu Arg Ser Leu  
 245 250 255

Glu Ile Leu Arg Ile Arg Lys Val Asn Gly Leu Ala Arg Ile Lys Gly  
 260 265 270

Leu Lys Asp Leu Leu Cys Ser Ser Thr Cys Lys Leu Arg Lys Phe Tyr  
 275 280 285

Ile Thr Glu Cys Pro Asp Leu Ile Glu Leu Leu Pro Cys Glu Leu Gly  
 290 295 300

Gly Gln Thr Val Val Val Pro Ser Met Ala Glu Leu Thr Ile Arg Asp  
 305 310 315 320

Cys Pro Arg Leu Glu Val Gly Pro Met Ile Arg Ser Leu Pro Lys Phe  
 325 330 335

Pro Met Leu Lys Lys Leu Asp Leu Ala Val Ala Asn Ile Thr Lys Glu  
 340 345 350

Glu Asp Leu Asp Ala Ile Gly Ser Leu Glu Glu Leu Val Ser Leu Glu  
 355 360 365

Leu Glu Leu Asp Asp Thr Ser Ser Gly Ile Glu Arg Ile Val Ser Ser  
 370 375 380

Ser Lys Leu Gln Lys Leu Thr Thr Leu Val Val Lys Val Pro Ser Leu  
 385 390 395 400

Arg Glu Ile Glu Gly Leu Glu Glu Leu Lys Ser Leu Gln Asp Leu Tyr  
 405 410 415

Leu Glu Gly Cys Thr Ser Leu Gly Arg Leu Pro Leu Glu Lys Leu Lys  
 420 425 430

Glu Leu Asp Ile Gly Gly Cys Pro Asp Leu Thr Glu Leu Val Gln Thr  
 435 440 445

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Val Val Ala Val Pro Ser Leu Arg Gly Leu Thr Ile Arg Asp Cys Pro  
 450 455 460

Arg Leu Glu Val Gly Pro Met Ile Gln Ser Leu Pro Lys Phe Pro Met  
 465 470 475 480

Leu Asn Glu Leu Thr Leu Ser Met Val Asn Ile Thr Lys Glu Asp Glu  
 485 490 495

Leu Glu Val Leu Gly Ser Leu Glu Glu Leu Asp Ser Leu Glu Leu Thr  
 500 505 510

Leu Asp Asp Thr Cys Ser Ser Ile Glu Arg Ile Ser Phe Leu Ser Lys  
 515 520 525

Leu Gln Lys Leu Thr Thr Leu Ile Val Glu Val Pro Ser Leu Arg Glu  
 530 535 540

Ile Glu Gly Leu Ala Glu Leu Lys Ser Leu Arg Ile Leu Tyr Leu Glu  
 545 550 555 560

Gly Cys Thr Ser Leu Glu Arg Leu Trp Pro Asp Gln Gln Gln Leu Gly  
 565 570 575

Ser Leu Lys Asn Leu Asn Val Leu Asp Ile Gln Gly Cys Lys Ser Leu  
 580 585 590

Ser Val Asp His Leu Ser Ala Leu Lys Thr Thr Leu Pro Pro Arg Ala  
 595 600 605

Arg Ile Thr Trp Pro Asp Gln Pro Tyr Arg  
 610 615

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## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 90 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 1..90

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GTG	TGT	TGT	TTT	TCA	GCT	GTT	CAT	ATG	AAG	GTG	TGT	TAT	CTT	CTT	ATT	48
Val	Cys	Cys	Phe	Ser	Ala	Val	His	Met	Lys	Val	Cys	Tyr	Leu	Leu	Ile	
1				5					10					15		
TGT	TCT	TCA	TAT	TTC	TGT	TTT	AAT	CTG	CTG	TCA	GAT	GGC	TG			90
Cys	Ser	Ser	Tyr	Phe	Cys	Phe	Asn	Leu	Leu	Ser	Asp	Gly				
				20				25				30				

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 29 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Val	Cys	Cys	Phe	Ser	Ala	Val	His	Met	Lys	Val	Cys	Tyr	Leu	Leu	Ile
1				5					10				15		
Cys	Ser	Ser	Tyr	Phe	Cys	Phe	Asn	Leu	Leu	Ser	Asp	Gly			
				20				25							

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## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 8 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

(xi(i)) /: Alternative amino acid

Gly Xaa Xaa Xaa Xaa Gly Lys Thr / Ser  
1 5

## (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 9 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Gly Met Gly Gly Ile Gly Lys Thr Thr  
1 5

## (2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 12 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Gly Leu Tyr Gly Met Gly Gly Ile Gly Lys Thr Thr  
1 5 10

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## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 840 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
AAAAAAAAAT TATAAGTTAA CAATTATACG TATGTTCCCTA ACAATTGCTA AATAAATATT      60
ATCTCATCCT TCACTCCATT TTAGCAATTG TTAAAATTTT GGAATCGATA AATCTCGATC      120
CACTAACCAC TTCAAAATAG AAAAATAATG CGTAGCACAC AAACAACAGG ATTGGTGGCC      180
CCTTGATCAT GTAGCCAATT AGGTGATTCT TGTGGACAAG TAGAACTAGT TGTACTCGCT      240
AACCGGTCCG ATTTGAAATT TGACCCAGGT TTCGAAATTC TAACCAACCC AAATGGTTGA      300
CTGACTTG TG GGCCTGCATT ATTTGTAGAT AAGAAAGAGT TTACAAATGG TTGGCTTACA      360
TTGAATTTGT GGAGCAATAA TTGAGTTTTT GAATGGAGGA TACTGAAAGC CAGTGGGAGC      420
TCAGAAGTAA GGGATAAGGC AAGAAGCAGA GGAGCAGAGA GAAGAACCAG CAAAGCACAT      480
GCAAATTGAA GCAGGCAAGA ACAGTTACTG GAAATTCATT TATCTCTGCT TTCAATTTCT      540
ATCCTTCAGA TCATTTCTGC TCAATTGAAT CACTAGTCAT GAGTTATTTG AGAGAAGTTG      600
CTACTGCTGT TGCCTTGCTT CTCCCTTTCA TTCTTCTCAA CAAGTTTTGG AGACCAAATT      660
CCAAAGACTC AATCGTCAAC GATGATGACG ATTCAACATC TGAAGTTGAT GCCATATCCG      720
ACTCCACAAA TCCCTCTGGT TCATTTCCCT CCGTGGAGTA TGAAGTGT TTGAGTTTCA      780
GGGGTCCAGA TACTCGTGAA CAGTTCACCG ATTTCTATA TCAGTCTCTC CGTCGCTATA      840
```

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## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 150 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```

Pro Asp Leu Thr Glu Leu Val Gln Thr Val Val Ala Val Pro Ser Leu
1           5           10           15

Arg Gly Leu Thr Ile Arg Asp Cys Pro Arg Leu Glu Val Gly Pro Met
20           25           30

Ile Gln Ser Leu Pro Lys Phe Pro Met Leu Asn Glu Leu Thr Leu Ser
35           40           45

Met Val Asn Ile Thr Lys Glu Asp Glu Leu Glu Val Leu Gly Ser Leu
50           55           60

Glu Glu Leu Asp Ser Leu Glu Leu Thr Leu Asp Asp Thr Cys Ser Ser
65           70           75           80

Ile Glu Arg Ile Ser Phe Leu Ser Lys Leu Gln Lys Leu Thr Thr Leu
85           90           95

Ile Val Glu Val Pro Ser Leu Arg Glu Ile Glu Gly Leu Ala Glu Leu
100          105          110

Lys Ser Leu Arg Ile Leu Tyr Leu Glu Gly Cys Thr Ser Leu Glu Arg
115          120          125

Leu Trp Pro Asp Gln Gln Gln Leu Gly Ser Leu Lys Asn Leu Asn Val
130          135          140

Leu Asp Ile Gln Gly Cys
145          150

```

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## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Ile Leu Val Val Leu Asp Asp Val Asp  
1 5

**CLAIMS**

1. An isolated nucleic acid molecule comprising a sequence of nucleotides which confers or otherwise facilitates disease resistance in plants.
2. An isolated nucleic acid molecule according to claim 1 wherein the disease resistance is rust resistance.
3. An isolated nucleic acid molecule according to claim 2 comprising a sequence of nucleotides as set forth in SEQ ID NO:1 or having at least 45% similarity to all or part thereof.
4. An isolated nucleic acid molecule according to claim 2 comprising a sequence of nucleotides encoding or complementary to a sequence of nucleotides encoding the amino acid sequence set forth in SEQ ID NOs:2 to 5 or having at least 45% similarity to all or part thereof.
5. An isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a peptide, polypeptide or protein which is capable of interacting with an avirulence gene product on a flax rust.
6. An isolated nucleic acid molecule according to claim 5 wherein the nucleic acid molecule corresponds to an allele of rust resistance gene L or M.
7. An isolated nucleic acid molecule according to claim 5 or 6 wherein the nucleic acid molecule corresponds to an L6 allele of rust resistance gene L and the corresponding avirulence gene is AL6.
8. An isolated nucleic acid molecule according to claim 5 or 6 wherein the nucleic acid molecule corresponds to the L2 allele of the rust resistance gene L and the corresponding avirulence gene is AL2.
9. An isolated nucleic acid molecule according to claim 5 or 6 wherein the nucleic acid molecule corresponds to the L10 allele of the rust resistance gene L and the corresponding avirulence gene is AL10.



10. An isolated nucleic acid molecule which:
  - (i) confers rust resistance in a plant; and
  - (ii) is capable of hybridizing under low stringency conditions to the nucleic acid molecule defined in SEQ ID NO:1.
11. An isolated nucleic acid molecule according to claim 10 wherein the nucleic acid molecule is capable of hybridizing to the nucleic acid defined in SEQ ID NOs:2 to 5 under medium stringent conditions.
12. An isolated nucleic acid molecule according to claim 10 wherein the nucleic acid molecule is capable to hybridizing to the nucleic acid defined in SEQ ID NO:11 under high stringent conditions.
13. An isolated nucleic acid molecule according to claim 10 or 11 or 12 having a nucleotide sequence substantially corresponding to the nucleotide sequence set forth in SEQ ID NO:1 or having at least 80% similarity to all or part thereof.
14. An isolated nucleic acid molecule according to claim 1 or 5 or 10 isolatable from a flax plant.
15. An isolated nucleic acid molecule according to claim 1 or 5 or 10 isolatable from a species of *Linum* or a soybean, sunflower, cereal, or wild varieties of wheat or barley.
16. An isolated nucleic acid molecule according to claim 15 isolatable from *Linum marginale*.
17. A method for identifying a rust resistance genetic sequence in a plant, said method comprising contacting genomic DNA or cDNA or mRNA from said plant with a hybridizing effective amount of a genetic sequence conferring or facilitating rust resistance or part thereof and then detecting said hybridization.
18. A method according to claim 17 wherein the plant is flax.

19. A method according to claim 17 wherein the plant is a species of *Linum*, soybean, sunflower, cereal, or a wild variety of wheat or barley.
20. A method according to claim 17 wherein the plant is *Linum marginale*.
21. A method according to claim 17 wherein the rust resistance genetic sequence is as set forth in SEQ ID NO:1 or is a portion or part thereof encoding rust resistance or has at least 60% similarity thereto.
22. A method according to claim 17 wherein the rust resistance genetic sequence encodes an amino acid sequence substantially as set forth in SEQ ID NOs:2 to 5 or having at least 60% similarity thereto.
23. An isolated nucleic acid molecule comprising a sequence of nucleotides which:
- (i) confers or otherwise facilitates disease resistance in plants; and
  - (ii) encodes a product having at least one leucine rich region and/or comprises a p-loop at the 5' end of the nucleic acid molecule which encodes the amino acid sequence:  
GXXXXGKT/S,  
where X is any amino acid residue.
24. An isolated nucleic acid molecule according to claim 23 wherein the leucine rich region is a leucine rich repeat encoded by the 3' end of the molecule.
25. A nucleic acid molecule according to claim 23 or 24 wherein the leucine region comprises the amino acid sequence:  
PDLIELLPCELGGQTVV.VPSMAELTIRDCPRLEVGP MIRSLPKF PMLKK  
LDLAVANITKEEDLDAIGSLEELVSLELELDDTSSGIERIVSSSKLQKLT  
TLVVKVPSLREIEGLEELKSLQDLYLEGCTSLGRL.....PLEKLKE  
LDIGGC  
or having at least 60% similarity to all or part thereof.
26. A nucleic acid molecule according to claim 23 wherein the p-loop sequence encodes the amino acid residues:  
GMGGIGKTT

27. A nucleic acid molecule according to claim 23 wherein the p-loop sequence encodes the amino acid residues:

GLYGMGGIGKTT

28. A nucleic acid molecule according to claim 23 wherein the disease resistance is rust resistance.

29. A nucleic acid molecule according to claim 28 wherein the plant is a crop plant.

30. A nucleic acid molecule according to claim 29 wherein the plant is wheat or barley.

31. A nucleic acid molecule according to claim 23 comprising a nucleotide sequence as set forth in SEQ ID NO:1 or encoding an amino acid sequence as set forth in SEQ ID NOs:2 to 5 or having at least 60% similarity to all or part thereof.

32. A modular resistance gene comprising heterologous genetic sequences from at least two nucleic acid molecules encoding disease resistance in plants.

33. A modular resistance gene according to claim 32 wherein the disease resistance is rust resistance.

34. A modular resistance gene according to claim 33 comprising all or part of genetic sequences encoding leucine rich regions from alleles of at least two rust resistance genes selected from L and M.

35. A modular resistance gene according to claim 34 comprising all or part of genetic sequences encoding leucine rich regions from alleles of at least two rust resistance genes selected from L6, L2 and L10.

36. A transgenic plant carrying a non-indigenous genetic sequence conferring or otherwise facilitating rust resistance in said plant.

37. A transgenic plant according to claim 36 wherein the non-indigenous sequence comprises all or part of the nucleotide sequence set forth in SEQ ID NO:1 or encodes an amino acid sequence as set forth in SEQ ID NOs:2 to 5 or having at least 60% similarity to all or part thereof.

38. A transgenic plant according to claim 36 or 37 wherein said plant is wheat or barley.

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13 / 19

FIG 1

## FIGURE 1

a.a.

nt.

GAGCTCAGAAAGTAAGGGATAAGGCAAGCAAGCAGAGGAGCAGAGAGAAGAACCAAGC 60  
 ACATGCAAAATTGAAGCAGGCAAGAACAGTTACTGTGAATTCATTATCTCTGCTTTCAAT 120  
 TTCTATCCCTTCAGATCATTTCTGCTCAATTGAATCACTAGTCATGAGTTATTGAGAGAA 180  
 GTTGCTACTGCTGTGCTTGCTTCTCCCTTTCATTCTCTCAACAAGTTTGGAGACCA 240  
 V A T A V A L L L P F I L L N K F W R P  
 AATCCAAAGACTCAATCGTCAACGATGATGACGATTCAACATCTGAAGTTGATGCCATA 300  
 N S K D S I V N D D D S T S E V D A I  
 TCCGACTCCACAAATCCCTCTGGTTCAATTCCTCCCTCCGTGGAGTATGAAGTGTTTTGAGT 360  
 S D S T N P S G S F P S V E Y E V F L S  
 TTCAGGGGTCCAGATACTCGTGAACAGTTCACCGATTTCCTATATCAGTCTCTCCGTCGC 420  
 F R G P D T R E Q F T D F L Y Q S L R R  
 TATAAGATTCACTTTTAGGGACGACGATGAGCTACTCAAGGAAAAGAAATAGGGCCC 480

1

7

27

47

67

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FIGURE 1

87 Y K I H T F R D D E L L K G K E I G P  
AACCTCCTACGAGCAATTGATCAGTCCAAAATTACGTCCCGATCATATCGAGCGGATAT 540

107 N L L R A I D Q S K I Y V P I I S S G Y  
GCTGATAGTAAGTGGTGTCTTATGGAGCTTGCTGAAATTGTGAGACGTCAAGAGGAGGAC 600

127 A D S K W C L M E L A E I V R R Q E E D  
CCTCGACGCATCATACTTCCTATTTTATATGGTGATCCAAAGTGACGTACGACATCAG 660

147 P R R I I L P I F Y M V D P S D V R H Q  
ACTGGATGTTATAAAAAGCATTTTCGAAAACACGCAATAAATTGATGGACAGACCATA 720

167 T G C Y K K A F R K H A N K F D G Q T I  
CAAAACTGGAAGATGCTCTAAAGAAGTCTGGAGACTTAAAGGATGCGACATCGGAAAG 780

187 Q N W K D A L K K V G D L K G W H I G K  
AATGACAAGTATGTAATCCTCATCCTAGCCCTTTATCATTCAGTACAACCTTATTGTTT 840

207 N D K  
GTCTTGCATATACTTACTTGTGTTTATTGACTAACTTTCAAATTGCATATTAACATTCTAGG 900

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FIGURE 1

AAGATTAAATTGGACTCGGGTCACAACTGAACCATATTATTTACACGACAAAGCACGA 960  
 GCTATTCCCTAATGTAATTAAAGTTAAAAATGCTCAATAACGTGGAATATATACTTGTATAC 1020  
 AGGGGCAACAACTATAAATTGAAATATTATTTTCATATGAAGTTAACATCTCAAAAATGT 1080  
 ATTTAATACTTGTAGGCAGGGAGCTATAGCAGACAAAGTATCAGCAGATATATGGTCACA 1140  
 210 Q G A I A D K V S A D I W S H 4/19  
 CATAAGCAAGGAAAATCTCATTTTAGAAACCGATGAGTTGGTTGGAATTGATGATCACAT 1200  
 225 I S K E N L I L E T D E L V G I D D H I  
 AACAGCCGTATTAGAAAAAATTGAGTTTAGACTCTGAAAATGTGACAAATGGTCGGCCTTTA 1260  
 245 T A V L E K L S L D S E N V T M V G L Y  
 TGGTATGGGTGGAATAGCAAGACGACCACCTGCAAGCGGTTTATAACAAGATTCTTTC 1320  
 265 G M G G I G K T T T A K A V Y N K I S S  
 TTGTTTCGATTGTTGTTGTTTATTGACAAACATACGTGAAACACAAAGAGGATGGCGT 1380  
 285 C F D C C C F I D N I R E T Q E K D G V



FIGURE 1

TGTTGTTTGC AAAAGCTAGTATCTGAAATTTTGAGGATCGATTCCGGTTCGGTTGG 1440  
 305 V V L Q K K L V S E I L R I D S G S V G  
 ATTTAATAATGATAGTGGCGGACGGAAGACGATAAAGGAGAGAGTTTCGAGGTTCAAAAT 1500  
 325 F N N D S G G R K T I K E R V S R F K I  
 TCTTGTCGTTCTCGATGATGTGGATGAGAAAGTTTAAATTTGAAGATATGTTGGGAAGTCC 1560  
 345 L V V L D D V D E K F K F E D M L G S P  
 TAAAGATTTTATTCTCAAAGTAGATTTCATTATTACTTCAAGAAGTATGAGAGTTTGGG 1620  
 365 K D F I S Q S R F I I T S R S M R V L G  
 TACTTTGAATGAGAATCAATGCAAGTTGTATGAAGTTGGATCGATGAGCAAAACCACGTTTC 1680  
 385 T L N E N Q C K L Y E V G S M S K P R S  
 GCTTGAACTCTTCTCCAAGCATGCATTCAAAAAGAATAACGCCTCCATCGTATTATGAGAC 1740  
 405 L E L F S K H A F K K N T P P S Y Y E T  
 TCTAGCAAATGACGTCGTAGATACTACAGCAGGACTTCCATTGACTCTGAAGGTTATAGG 1800  
 425 L A N D V V D T T A G L P L T L K V I G

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FIGURE 1

ATCGCTTTTATTAAACAAGAGATTGCGGTTTGGGAAGACACGTTGGAACAATTACGTAG 1860

445 S L L F K Q E I A V W E D T L E Q L R R  
AACACTTAACCTTGATGAGGTTTATGATAGGCTAAAAATAAGTTATGATGCGTTGAACCC 1920

465 T L N L D E V Y D R L K I S Y D A L N P  
GGAGGCAAAAGAGATTTCCTTGGATATAGCTTGCTTCTTCATCGGACAAAATAAAGAAGA 1980

485 E A K E I F L D I A C F F I G Q N K E E  
ACCGTATTACATGTGGACCGACTGTAATTTTATCCAGCAAGTAATATTATTTTCTCAT 2040

505 P Y Y M W T D C N F Y P A S N I I F L I  
TCAAAGATGTATGATACAAGTTGGGGATGATGATGAGTTTAAATGCACGACCAACTTAG 2100

525 Q R C M I Q V G D D D E F K M H D Q L R  
AGATATGGGTAGAGAAATTGTGAGACGAGAGGATGTACTGCCGTGGAAGAGAGTAGAAT 2160

545 D M G R E I V R R E D V L P W K R S R I  
ATGGTCGGCAGAAGAAGGATGTATCTCTTGCTGAACAAAGGTATTCAGTTTATTATTA 2220

565 W S A E E G I D L L L N K K

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## FIGURE 1

AAATTAAATATTCATATATTATTACACATACTTTAAATCACATAACTCATTACGTTCTT 2280

CTCCAATTACAGGGATCAAGTAAAGTAAAGCAATTAGCATACCCCTGGGGTGTCAGTAT 2340

579 G S S K V K A I S I P W G V K Y  
GAGTTTAAGAGCGAATGTTTCTTGAATTTGTCTCAGAGTTGAGATACCTCCATGCAAGGGAA 2400

595 E F K S E C F L N L S E L R Y L H A R E  
GCCATGCTTACCGGAGATTTCACAACATCTTCTCCCGAATTAAAGTGGCTTGAGTTGCCA 2460

615 A M L T G D F N N L L P N L K W L E L P  
TTTACAAACATGGAGAGGATGATCCTCCTTTGACCAATTATACCATGAAAAATCTGATA 2520

635 F Y K H G E D D P P L T N Y T M K N L I  
ATTGTTATTCTTGAGCATAGCCACATAACGGCTGATGATTGGGAGGTTGGAGGCATATG 2580

655 I V I L E H S H I T A D D W G G W R H M  
ATGAAGGTGTGTTGTTTTCAGCTGTTCATATGAAGGTGTGTATCTTCTTATTGTTCT 2640

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FIGURE 1

675 M K [v c c f s a v h m k v c y l l i c s  
 TCATATTCTGTTTAAATCTGCTGTCAGATGGCTGAGAGGCTGAAAAGTTGTACGACTTGC 2700  
s y f c f n l l l s d g \*l  
 677 TTCAAACCTATAGTTTGTACGGAAGACGTTGTCGCCTTCTGACTGTGCGGCTTCCCAA 2760  
 M A E R L K V R L A  
 688 S N Y S L Y G R R V R L S D C W R F P K 8/19 2820  
 AAGCATGAGGTATTATCCATGACTGCGATAGAAAATGATGAAGTTGATATTTGGGAGTT  
 708 S I E V L S M T A I E M D E V D I G E L 2880  
 AAAGAAGCTAAAGACGTTGGTTCTGAAATTCTGTCCAATACAAAAGATAAGTGGGGGAAC  
 728 K K L K T L V L K F C P I Q K I S G G T 2940  
 CTTTGGTATGTTGAAGGACTTCGAGAGCTTTGTCTCGAATTCAACTGGGGGACAAATTT  
 748 F G M L K G L R E L C L E F N W G T N L 3000  
 GAGAGAGGTAGTTGCCGATATTGGTCAACTTTTCATCTCTCAAGTCTTGAAAACAACCGG

FIGURE 1

768 R E V V A D I G Q L S S L K V L K T T G  
AGCTAAGGAGTCGAGATTAATGAATTCCATTAGGTTGAAGGAGTTATCCACTTCATC 3060

788 A K E V E I N E F P L G L K E L S T S S  
TCGGATTCCGAATCTTTCACAGTTGTTGGATTGGAGTACTGAAGGTTTATGATTGCAA 3120

808 R I P N L S Q L L D L E V L K V Y D C K  
GGATGGATTGACATGCCCTCCTGCTAGTCCGAGTGAAGATGAAGTAGTGTGTGGTGAA 3180

828 D G F D M P P A S P S E D E S S V W K  
GGTATCCAAGTTGAAGTCTTTTGCAACTCGAGAAGACAAGAATCAATGTCAACGTTGTGGA 3240

848 V S K L K S L Q L E K T R I N V N V V D  
TGATGCTTCTCCGGTGGTCACTCCCTCGTTACTTACTACCAACATCCCCTAACCTATCT 3300

868 D A S S G G H L P R Y L L P T S L T Y L  
TAAATTTATCAGTGTACAGAACCAACGTTGGCTTCCAGGAATAGAGAACTTGGAGAATTT 3360

888 K I Y Q C T E P T W L P G I E N L E N L  
GACTTCGCTGGAAGTCAACGACATCTTCCAAACTCTTGGAGGTGACTTGGATGGGCTACA 3420

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## FIGURE 1

908 T S L E V N D I F Q T L G G D L D G L Q  
AGGGTTGAGATCATTTGGAAATTCTTAGGATTCGGAAGTAAATGGTTAGCTCGGATCAA 3480

928 G L R S L E I L R I R K V N G L A R I K  
AGGGCTTAAGGATCTCTTGTTGTTCTTCTACCTGCAAGTTGCGGAAATTTTATATTACAGA 3540

948 G L K D L L C S S T C K L R K F Y I T E  
ATGCCCCGACCTCATTTGAGTTACTCCCATGCGAACTCGGCGGCCAAACAGTAGTAGTCCC 3600

968 C P D L I E L L P C E L G G Q T V V V P  
CTCTATGGCAGAACTGACCATTAGGGATTGTCCACGGCTGGAGGTTGGCCCGATGATAAG 3660

988 S M A E L T I R D C P R L E V G P M I R  
ATCACTCCCAAGTTCCCAATGCTAAAGAAAGTTGGACCTCGCGGTGGCAAAATATAACTAA 3720

1008 S L P K F P M L K K L D L A V A N I T K  
AGAGGAGGATCTGGATCGGATTGGATCCCTAGAGAGTTGTTAGTTTGGAGTTAGAGTT 3780

1028 E E D L D A I G S L E E L V S L E L E L  
AGACGATACATCTCCGGTATAGAGAGGATAGTTTCCCTCTTCGAAGCTGCAAAAGTTAAC 3840

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FIGURE 1

1048 D D T S S G I E R I V S S S K L Q K L T  
 TACTCGTAGTGAAGTGCCGAGTTTGCGGGGAGATTGAAGGCTTGAAGAGTTGAAGTC 3900  
  
 1068 T L V V K V P S L R E I E G L E E L K S  
 TTTACAAGATTGTATCTAGAGGGTTGCACGTCGTTGGGGAGACTACCCTGGAGAAGCT 3960  
  
 1088 L Q D L Y L E G C T S L G R L P L E K L  
 GAAGGAGCTAGACATTGGAGGATGCCCTGACCTCACTGAGTTAGTCCAAACAGTAGTAGC 4020  
  
 1108 K E L D I G G C P D L T E L V Q T V V A  
 AGTCCCCCTCTTTGAGAGGACTGACCATTAGGGATTGTCCACGCTGGAGGTTGGTCCAAT 4080  
  
 1128 V P S L R G L T I R D C P R L E V G P M  
 GATACAATCTCTTCCAAAGTTCCCAATGCTAAATGAATTGACGCTCTCGATGGTAAATAT 4140  
  
 1148 I Q S L P K F P M L N E L T L S M V N I  
 CACTAAGGAGGATGAGCTGGAGGTGCTTGGATCCCTAGAAAGAGTTGGATAGTTTGGAGTT 4200  
  
 1168 T K E D E L E V L G S L E E L D S L E L  
 AACGTTAGACGATACATGTTCCAGCATAGAGGATATCTTTCTTGTGCGAAGCTGCAAAA 4260

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## FIGURE 1

1188 T L D D T C S S I E R I S F L S K L Q K  
GTTAACTACTCATAGTGGAGGTGCCGAGTTTTCGGGAGATTGAAGGCTTTGCAGAGTT 4320

1208 L T T L I V E V P S L R E I E G L A E L  
GAAGTCTTTACGAATTTTGTTATCTAGAAGGATGCACGTCGTTGGAAAAGACTGTGGCCTGA 4380

1228 K S L R I L Y L E G C T S L E R L W P D  
TCAACAACAGTTGGTAGTCTGAAGAACCCTGAATGTGCTCGACATCCAAGGTTGTAAAG 4440

1248 Q Q Q L G S L K N L N V L D I Q G C K S  
CTTGAGTGTGACCATCTCTCTGCACTCAAGACCACCTCTACCGCCAGGCGAGGATAAC 4500

1268 L S V D H L S A L K T T L P P R A R I T  
ATGGCCCGATCAGCCCTACAGATGACGGTAGGAATTAATGCAAGTGACAGTGATGACATA 4560

1288 W P D Q P Y R \*  
GTTGTGATGGCTTGACCTGATCAACCTTGTTCTTTCATGGGGTTTCTCCCCAGTGAGAT 4620

CTTAATATCCAAAATTCTGGTTTGTTCAGAGGTTATATGGTTCAGTTTTCACCATAATA 4680

TTTTCTACGGCATAAGCGCAAACTACTTCTAGTATATAGATATAGGCAATATATATAACAT 4740

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## FIGURE 1

CCAACTCTGTTTACTCACTCCTCTCATCTTCTCACTCGATTATGTTCCATTTCCTAAAT 4800

CCATTATTCA TCGCCTTATATTCATAATTATGTAATTATT 4838

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FIG 2

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FIGURE 2

↓ HincII  
60  
AAAAAAAAT TATAAGTTAA CAATTATACG TATGTTCTTA ACAATTGCTA AATAAATATT  
120  
ATCTCATCCT TCACTCCATT TTAGCAATTG TTAAAATTTT GGAATCGATA AATCTCGATC  
180  
CACTAACCAC TTCAAAATAG AAAAATAATG CGTAGCACAC AAACAACAGG ATTGGTGGCC  
240  
CCTTGATCAT GTAGCCAATT AGGTGATTCT TGTGGACAAG TAGAACTAGT TGTACTCGCT  
↓ HincII  
300  
AACCGGTCCG ATTTGAAATT TGACCCAGGT TTCGAAATTC TAACCAACCC AAATGGTTGA  
360  
CTGACTTGTG GGCCTGCAAT ATTTGTAGAT AAGAAAGAGT TTACAAATGG TTGGCTTACA  
420  
TTGAATTGT GGAGCAATAA TTGAGTTTTT GAATGGAGGA TACTGAAAGC CAGTGGGAGC  
480  
TCAGAAGTAA GGGATAAGGC AAGAAGCAGA GGAGCAGAGA GAAGAACCAG CAAAGCACAT  
540  
GCAAATTGAA GCAGGCAAGA ACAGTTACTG GAAATTCATT TATCTCTGCT TTCAATTCTT  
600  
ATCCTTCAGA TCATTCTGC TCAATTGAAT CACTAGTCAT GAGTTATTG AGAGAAGTTG



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probe LU-2 detects

L6 + M region

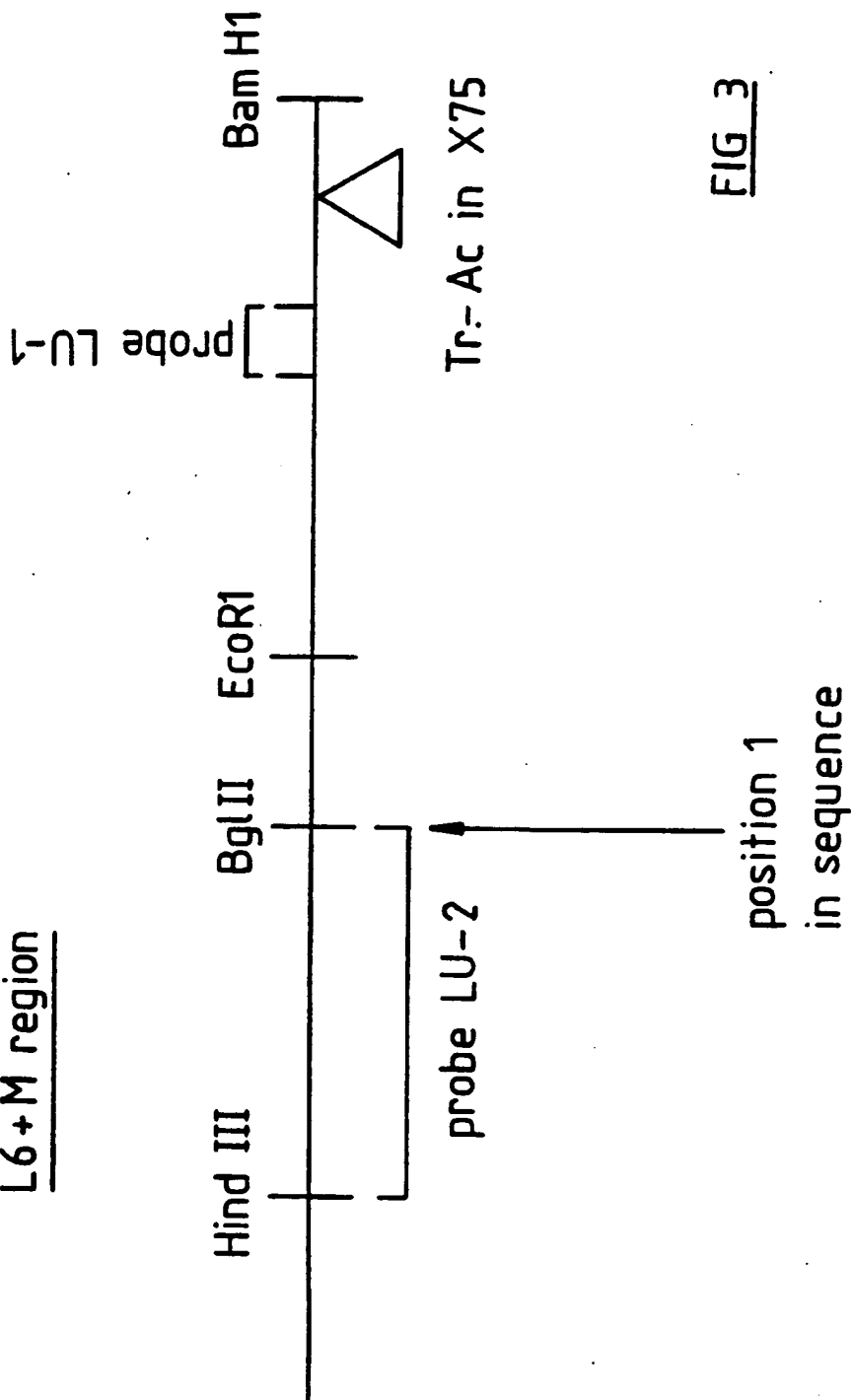


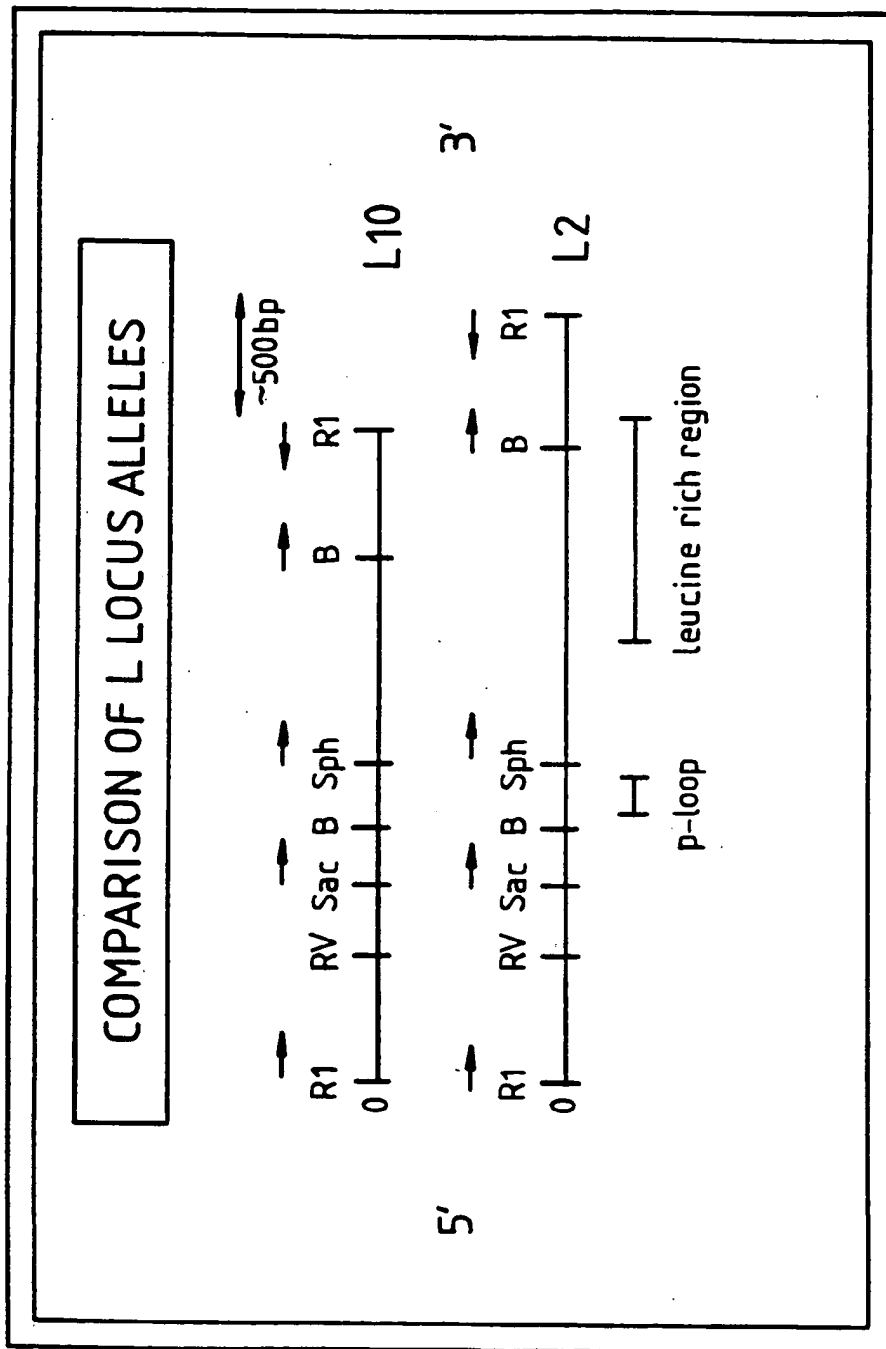
FIG 3

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FIGURE 4

969 PDLIELLPCELGGQTVV.VPSMAELTIRDPCPRLEVGP MIRSLPKFPMLKK 1017  
 |||.||: ||| |: ||| ||| ||| ||| ||| ||| ||| ..  
 1116 PDLTELV.....QTVVAVPSLRGLTIRDCPRLEVGP MIQSLPKFPMLNE 1159  
 1018 LDLAVANITKEEDLDAIGSLEELVSLELEDDTSSGIERIVSSSKLQKLT 1067  
 |.|.: ||| |: ||| ||| ||| |: ||| ||| ||| ||| |||  
 1160 LTL SMVNITKEDELEVLGSLEELDSLELTLD DDCSSIERISFLSKLQKLT 1209  
 1068 TLVVKVPSLREIEGLEELKSLQDLYLEGGCTSLGRL.....PLEKLKE 1109  
 |:|. ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| :|.|. .  
 1210 TLIVEVPSLREIEGLAELKSLRILYLEGGCTSLERLWPDQQQLGSLKNLNV 1259  
 1110 LDIGGC 1115  
 |||.||  
 1260 LDIQGC 1265

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**FIG 5**

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU 95/00240

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> Int. Cl. <sup>6</sup> C12N 15/29; C12Q 1/68  According to International Patent Classification (IPC) or to both national classification and IPC					
<b>B. FIELDS SEARCHED</b>  Minimum documentation searched (classification system followed by classification symbols) WPAT : CASM : Keywords as below  Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  Electronic data base consulted during the international search (name of data base, and where practicable, search terms used) WPAT : rust and C12N/IC or A01H/IC or C07K/IC CASM (1) : rust and gene; or DNA or nucleic acid; and resist; or protect; or avirulen; and plant and 1990-1995 (2) leucine rich and plant; and 1990-1995 and resist; or protect; or avirulen; (3) plant; and p(w) loop or leucine (s) rich BIOSIS : (1) search 1 above (years 1985-1995) (2) search 3 above STN search : GXXXXGKT; GXXXXGKS; GMGGIGKTT; GLEELKSL; GLYGMGGIGKTT; TTCGA and 1989-1995 and rust and resist:					
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>					
<b>Category*</b>	<b>Citation of document, with indication, where appropriate, of the relevant passages</b>	<b>Relevant to Claim No.</b>			
P,X	Science vol. 265 pages 1856-1860 (23 September 1994) A.F. Bent et al "RPS2 of <u>Arabidopsis thaliana</u> : A Leucine-Rich Repeat Class of Plant Disease Resistance Genes" (see whole document, in particular, column 2 page 1858 and figure 4)	1, 23			
<div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 45%;"> <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C.         </div> <div style="width: 45%;"> <input type="checkbox"/> See patent family annex.         </div> </div>					
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> <p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </td> <td style="width: 33%; vertical-align: top;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p> </td> <td style="width: 33%;"></td> </tr> </table>			<p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p>	
<p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p>				
Date of the actual completion of the international search 10 August 1995	Date of mailing of the international search report 11 August 1995 (11.08.95)				
Name and mailing address of the ISA/AU  AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA  Facsimile No. 06 2853929	Authorized officer <i>for Anadi Sardana</i> KAREN AYERS  Telephone No. (06) 2832082				



C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
P,X	Science, vol. 266 pages 789-793 (4 November 1994) P.A. Jones et al "Isolation of the tomato Cf.9 Gene for Resistance to <u>Cladosporium fulvum</u> by Transposon Tagging" (see whole document, in particular columns 2 and 3, page 791)	1
P,X	Cell, volume 78, pages 1101-1115 (September 23, 1994), S. Whitham et al "The Product of the Tobacco Mosaic Virus Resistance Gene N: Similarity to toll and the Interleukin-1 receptor (see whole document)	1
X Y	Bio/Technology volume 11 (9) (September 1993) pages 1048-1052, E. Truve et al "Transgenic potato plants expressing Mammalian 2'-5' oligoadenylate synthetase are protected from potato virus X Infection under field conditions " (see whole document)	1 32
X Y	Science, volume 258 (November 6, 1992) pages 985-987, G.S. Johal and S.P. Briggs, "Reductase activity encoded by the HM1 Disease Resistance Gene in Maize" (see whole document)	1 32
X Y	Science, volume 262 (November 26, 1993) pages 1432-1436, G.B. Martin et al "Map-Based cloning of a protein kinase gene conferring Disease resistance in Tomato" (see whole document)	1 32
A	Advances in Molecular Genetics of Plant-Microbe Interactions, volume 14 (1993), pages 469-475, A. Pryor "Transposon tagging of a rust resistance gene in maize" (see whole document)	1-38
A	Advances in Molecular Genetics of Plant-Microbe Interactions, volume 14 (1993), pages 511-515, H.J. Newbury et al "Mutagenesis of a race-specific rust resistance gene in <u>Antirrhinum Majus</u> using a transposon-tagging protocol" (see whole document)	1-38
T	Cell, volume 80 (February 10, 1995), pages 363-366, J.L. Dangl, "Pièce de Résistance: Novel Classes of Plant Disease Resistance Genes". (See whole document, particularly columns 2 and 2, page 363)	1-38